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 NEWS  5  NOV 03   JAPIO enhanced with IPC 8 features and functionality
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                   8.01c now available
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                   additional databases
 NEWS  9  NOV 20   CA/CAplus to MARPAT accession number crossover limit increased
                   to 50,000
 NEWS 10  DEC 01   CAS REGISTRY updated with new ambiguity codes
 NEWS 11  DEC 11   CAS REGISTRY chemical nomenclature enhanced
 NEWS 12  DEC 14   WPIDS/WPINDEX/WPIX manual codes updated
 NEWS 13  DEC 14   GBFULL and FRFULL enhanced with IPC 8 features and
                   functionality
 NEWS 14  DEC 18   CA/CAplus pre-1967 chemical substance index entries enhanced
                   with preparation role
 NEWS 15  DEC 18   CA/CAplus patent kind codes updated
 NEWS 16  DEC 18   MARPAT to CA/CAplus accession number crossover limit increased
                   to 50,000
 NEWS 17  DEC 18   MEDLINE updated in preparation for 2007 reload
 NEWS 18  DEC 27   CA/CAplus enhanced with more pre-1907 records
 NEWS 19  JAN 08   CHEMLIST enhanced with New Zealand Inventory of Chemicals

 NEWS EXPRESS  NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT
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=> s recombinant Escherichia coli
L1         8476 RECOMBINANT ESCHERICHIA COLI

=> s l1 and heat-killed
L2            0 L1 AND HEAT-KILLED

=> s l1 and modified allergen
L3            0 L1 AND MODIFIED ALLERGEN

=> s Escherichia coli
L4      1162694 ESCHERICHIA COLI

=> s l4 and recombinant allergen
L5          462 L4 AND RECOMBINANT ALLERGEN

=> s l5 and modified
L6           19 L5 AND MODIFIED

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L8             9 DUP REMOVE L6 (10 DUPLICATES REMOVED)

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L8   ANSWER 1 OF 9     MEDLINE on STN
2006548078.    PubMed ID: 16956374.   Ca2+-binding allergens from olive pollen
     exhibit biochemical and immunological activity when expressed in stable
     transgenic Arabidopsis. Ledesma Amalia; Moral Veronica; Villalba Mayte;
     Salinas Julio; Rodriguez Rosalia. (Dpto. Bioquimica y Biologia Molecular
     I, Universidad Complutense, Madrid, Spain. ) The FEBS journal, (2006 Oct)
     Vol. 273, No. 19, pp. 4425-34.  Electronic Publication: 2006-09-05.
     Journal code: 101229646. ISSN: 1742-464X. Pub. country: England: United
     Kingdom. Language: English.
AB   Employing transgenic plants as alternative systems to the conventional
     Escherichia coli, Pichia pastoris or baculovirus hosts
     to produce recombinant allergens may offer the
     possibility of having available edible vaccines in the near future.  In
     this study, two EF-hand-type Ca2+-binding allergens from olive pollen, Ole
     e 3 and Ole e 8, were produced in transgenic Arabidopsis thaliana plants.
     The corresponding cDNAs, under the control of the constitutive CaMV 35S
     promoter, were stably incorporated into the Arabidopsis genome and encoded
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recombinant proteins, AtOle e 3 and AtOle e 8, which exhibited the molecular properties (i.e. MS analyses and CD spectra) of their olive and/or E. coli counterparts. Calcium-binding assays, which were carried out to assess the biochemical activity of AtOle e 3 and AtOle e 8, gave positive results. In addition, their mobilities on SDS/PAGE were according to the conformational changes derived from their Ca2+-binding capability. The immunological behaviour of Arabidopsis-expressed proteins was equivalent to that of the natural- and/or E. coli-derived allergens, as shown by their ability to bind allergen-specific rabbit IgG antiserum and IgE from sensitized patients. These results indicate that transgenic plants constitute a valid alternative to obtain allergens with structural and immunological integrity not only for scaling up production, but also to develop new kind of vaccines for human utilization.

L8 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1

2006223169. PubMed ID: 16630158. Structural, immunological and functional properties of natural recombinant Pen a 1, the major allergen of Brown Shrimp, Penaeus aztecus. Reese G; Schicktanz S; Lauer I; Randow S; Luttkopf D; Vogel L; Lehrer S B; Vieths S. (Paul-Ehrlich-Institut, Department of Allergology, Langen, Germany.. reege@pei.pe) . Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, (2006 Apr) Vol. 36, No. 4, pp. 517-24. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Recombinant allergens are considered the basis for new diagnostic approaches and development of novel strategies of allergen-specific immunotherapy. As Pen a 1 from brown shrimp Penaeus aztecus is the only major allergen of shrimp and binds up to 75% of all shrimp-specific IgE antibodies this molecule may be an excellent model for the usage of allergens with reduced IgE antibody-binding capacity for specific immunotherapy. AIM: The aim was to clone, express and characterize a full-length recombinant Pen a 1 molecule and compare it with natural Pen a 1 in regard to structural and immunological parameters such as IgE antibody capacity and ability to induce IgE-mediated mediator release. METHODS: Total RNA was isolated from P. aztecus and a rapid amplification of cDNA ends (5' RACE) was performed to obtain full-length cDNA coding for Pen a 1. Using a gene-specific primer, PCR was performed and full-length cDNA was cloned and sequenced. Recombinant His-tagged Pen a 1 was isolated from Escherichia coli under native conditions by immobilized metal affinity chromatography. Secondary structure of natural and recombinant Pen a 1 was compared by circular dichroism (CD) spectroscopy, and the IgE antibody-binding capacity evaluated by RAST. The allergenic potency was tested by the capability of natural and recombinant Pen a 1 to induce mediator release in a murine and human in vitro model of IgE-mediated type I allergy. RESULTS: The deduced amino-acid sequence was 284 residues long and amino-acid sequence identities with allergenic and non-allergenic tropomyosins ranged from 80% to 99% and 51% to 58%, respectively. The analysis of the secondary structure of natural and recombinant Pen a 1 by CD spectroscopic analysis showed that both nPen a 1 and rPen a 1 had alpha-helical conformation that is typical for tropomyosin. The IgE antibody binding capacities of nPen a 1 and r Pen a1 were found to be essentially identical by RAST. The mediator release experiments using both wild-type and humanized rat basophilic leukaemia 30/25 cells showed that rPen a 1 and nPen a 1 induced a similar level of mast cell activation. CONCLUSIONS: Recombinant Pen a 1 and natural Pen a 1 are structurally and immunologically identical and rPen a 1 may be used as the basis for component-resolved diagnosis and the generation of modified shrimp tropomyosin for allergen-specific immunotherapy. The results of the animal studies indicate that C3H/HeJ mice that were sensitized with shrimp extract in combination with cholera toxin as adjuvant may be a suitable model to study shrimp allergy.

L8 ANSWER 3 OF 9 EMBASE

2006254921 EMBASE Recombinant expression systems for allergen vaccines. Singh

M.B.; Bhalla P.L.. M.B. Singh, Institute of Land and Food Resources, The University of Melbourne, Parkville, Vic. 3010, Australia. mohan@unimelb.edu.au. Inflammation and Allergy - Drug Targets Vol. 5, No. 1, pp. 53-59 2006.
Refs: 60.
ISSN: 1871-5281. Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 20060615. Last Updated on STN: 20060615

AB Allergen immunotherapy of future is likely to be based on allergy vaccines that contain engineered allergens modified to abolish or substantially reduce their IgE-binding activity in order to remove the risk of unwanted anaphylactic responses. The development of efficient systems for the production of recombinant allergens in sufficient quantities is requirement for establishing use of engineered allergens as components of allergy vaccines. This review outlines relative advantages and disadvantages of various heterologous systems for production of recombinant allergens. Microbial systems are most convenient and cost effective platforms for the production of recombinant allergens. However, lack of post-translational processing implies that some allergens have to be expressed in eukaryotic systems for proper folding and post-translational modifications such as glycosylation. Yeast systems can yield high levels of recombinant allergens but often are associated with hyper- glycosylation problems. Mammalian cell culture systems offer suitable post -translational modifications but are nearly hundred fold more expensive than microbial systems. The use of plants as bio-factories for production of recombinant allergens is emerging as a very attractive option as plants-based production system offer several advantages over other expression systems such as post translational processing of proteins, low production costs, scale up ability and enhanced safety due to absence of animal or human pathogens. .COPYRGT. 2006 Bentham Science Publishers Ltd.

L8 ANSWER 4 OF 9 MEDLINE on STN

2004221551. PubMed ID: 15119037. Expression of allergens in E. coli and plants--benefits and drawbacks. Breiteneder Heimo; Wagner Birgit. (Dept. of Pathophysiology, University of Vienna, Wahringer Gurtel 18-20, A-1090 Vienna.) Arbeiten aus dem Paul-Ehrlich-Institut (Bundesamt fur Sera und Impfstoffe) zu Frankfurt a.M, (2003) No. 94, pp. 178-87. Ref: 51. Journal code: 8912864. ISSN: 0936-8671. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Recombinant allergens are quickly becoming the reagents of choice for diagnosis and therapy of type I allergic diseases. Consequently, the different methods for the production of recombinant proteins that are available today are of great interest to allergologists. Without doubt, bacterial expression will continue to play a pivotal role. In addition, plant-based expression systems will be needed to overcome problems inherent in the E. coli systems and to allow the production of glycoallergens or allergens of more complex folding.

L8 ANSWER 5 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2003:74387 The Genuine Article (R) Number: 632HU. Art v 1, the major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain. Himly M; Jahn-Schmid B; Dedic A; Kelemen P; Wopfner N; Altmann F; van Ree R; Briza P; Richter K; Ebner C; Ferreira F (Reprint). Salzburg Univ, Inst F Genet U Allg Biol, Hellbrunnerstr 34, A-5020 Salzburg, Austria (Reprint); Salzburg Univ, Inst Genet & Gen Biol, A-5020 Salzburg, Austria; Univ Vienna, Inst Pathophysiol, A-1090 Vienna, Austria; Univ Agr Vienna, Inst Chem, A-1190 Vienna, Austria; Sanquin Res CLB, Dept Immunopathol, NL-1066 CX Amsterdam, Netherlands. fatima.ferreira @mh.sbg.ac.at. FASEB JOURNAL (NOV 2002) Vol. 16, No. 13, pp. 106-+. ISSN: 0892-6638. Publisher: FEDERATION AMER SOC EXP BIOL, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In late summer, pollen grains originating from Compositae weeds (e.g., mugwort, ragweed) are a major source of allergens worldwide. Here, we report the isolation of a cDNA clone coding for Art v 1, the major allergen of mugwort pollen. Sequence analysis showed that Art v 1 is a secreted allergen with an N-terminal cysteine-rich domain homologous to plant defensins and a C-terminal proline-rich region containing several (Ser/Ala)(Pro)(2-4) repeats. Structural analysis showed that some of the proline residues in the C-terminal domain of Art v 1 are posttranslationally modified by hydroxylation and O-glycosylation. The O-glycans are composed of 3 galactoses and 9-16 arabinoses linked to a hydroxyproline and represent a new type of plant O-glycan. A 3-D structural model of Art v 1 was generated showing a characteristic "head and tail" structure. Evaluation of the antibody binding properties of natural and recombinant Art v 1 produced in Escherichia coli revealed the involvement of the defensin fold and posttranslational modifications in the formation of epitopes recognized by IgE antibodies from allergic patients. However, posttranslational modifications did not influence T-cell recognition. Thus, recombinant nonglycosylated Art v 1 is a good starting template for engineering hypoallergenic vaccines for weed-pollen therapy.

L8 ANSWER 6 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2000:267299 The Genuine Article (R) Number: 297YX. Recombinant allergens: application to diagnostic and therapeutic perspectives. Pauli G (Reprint); Deviller P. Hop Univ Strasbourg, Serv Pneumol, BP 426, F-67091 Strasbourg, France (Reprint); Hop Univ Strasbourg, Serv Pneumol, F-67091 Strasbourg, France. REVUE DES MALADIES RESPIRATOIRES (FEB 2000) Vol. 17, No. 1BIS, pp. 293-303. ISSN: 0761-8425. Publisher: MASSON EDITEUR , 120 BLVD SAINT-GERMAIN, 75280 PARIS 06, FRANCE. Language: French. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Techniques of generic engineering applied to allergens have enabled the production of recombinant allergens. The validation of recombinant allergens implies that their immunological activity and their identity with natural allergens might be confirmed by in vitro and in vivo techniques carried out on a sufficiently large number of allergic subjects. Currently available results for the principal pneumoallergens are reported. Thus the work of validating recombinant allergen BeTv1 has been confirmed by in vitro tests and also by skin tests and nasal and bronchial provocation tests. The association of four recombinant allergens of phleole has enabled the detection in vitro of sensitisation to germinated pollens in 94.5% of patients. For mites the validity of group 2 recombinant allergens has been confirmed. A system enabling the expression of glycosylation of recombinant proteins was necessary to validate recombinant proteins in group 1 allergens. The recombinant allergen Blot5 is recognised as bring effective in the detection of sensitization to Blomia tropicalis, a domestic allergen in sub tropical countries. The recombinant allergens Bla g 4 and Bla g 5 have been tested in vitro and in vivo and reactions were positive in nearly 50% of subjects sensitive to cockroaches. The recombinant Asp f 1 has been tested in subjects suffering from allergic bronchopulmonary aspergillosis and is positive in 60-85% of cases.

Some studies are available for recombinant allergens of certain animal antigens (Equ c 1, Bos d 2). The consequences of clarifying recombinant allergens are then analysed : obtaining better standardised allergens for diagnostic tests, studying the spectrum of specificities of IgE induced by an allergen, the quantification of specific IgE, a better approach to mixed allergies with the help of recombinant allergens of the principal mixed allergens. Some recent progress has led to the production of modified recombinant allergens : the synthesis of recombinant polypeptides corresponding to T epitopes, the production of isoform recombinant allergens with

reduced allergenic activity, the production of recombinant allergens of modified allergenic molecules by directed mutations and the production of recombinant fragments of allergenic molecules. The use of modified recombinant allergens is a way of permitting research which would, in the future, lead to new modalities of specific immunotherapy.

L8 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 2

1999242424. PubMed ID: 10224369. The importance of recombinant allergens for diagnosis and therapy of IgE-mediated allergies. Kraft D; Ferreira F; Vrtala S; Breiteneder H; Ebner C; Valenta R; Susani M; Breitenbach M; Scheiner O. (Institute of General and Experimental Pathology, University of Vienna, Austria.) International archives of allergy and immunology, (1999 Feb-Apr) Vol. 118, No. 2-4, pp. 171-6. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB In the past 10 years, a considerable number of cDNAs coding for allergens have been isolated and expressed. Intensive investigations showed that recombinant allergens and their respective natural counterparts possess comparable properties with respect to structure, function and interaction with the immune system. Recent studies documented that in vitro as well as in vivo diagnosis of IgE-mediated allergic diseases can be successfully improved by the application of recombinant allergens. In addition, new strategies for a safer specific immunotherapy (SIT) have been developed based on the knowledge of the primary structures of allergens. Naturally occurring isoforms of allergens as well as recombinant allergens with modified amino acid sequences show very low IgE binding capacity but strong T cell-stimulatory activity and represent possible candidates. In case of Bet v 1, the major birch pollen allergen, isoforms d, g and l and a Bet v 1a mutant, produced by site-directed mutagenesis resulting in 6 amino acid exchanges, fulfilled the above mentioned criteria. In a third approach, two adjacent peptides covering the entire Bet v 1a sequence were produced in an Escherichia coli expression system. These peptides contained most of the relevant T cell epitopes, but lost their IgE binding capacity and, thus, their ability to activate mast cells and basophils of sensitized patients. Our results suggest that allergen variants (isoforms, mutants, T cell epitope-containing peptides) may be used as 'hypoallergenic agents' in SIT.

L8 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:98094 The Genuine Article (R) Number: YT529. Diagnostic value of recombinant allergens.. Pauli G (Reprint). Hop Univ Strasbourg, Serv Pneumol, BP 426, F-67091 Strasbourg, France (Reprint); Hop Univ Strasbourg, Serv Pneumol, F-67091 Strasbourg, France. REVUE FRANCAISE D ALLERGOLOGIE ET D IMMUNOLOGIE CLINIQUE (1997) Vol. 37, No. 8, pp. 1093-1101. ISSN: 0335-7457. Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE. Language: French.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Genetic engineering techniques applied to allergens have allowed the production of recombinant allergens. Validation of recombinant allergens demands confirmation of their immunological activity and their identity with natural allergens by in vivo and in vitro techniques on a sufficiently large number of allergic subjects. The results currently available for the main respiratory allergens ar reported. For example, the validity of the birch recombinant allergen Bet v 1 was confirmed by in vitro tests, but also by skin tests and nasal and bronchial challenge tests. The combination of four recombinant allergens of timothy allowed the in vitro detection of sensitization to Graminaceae pollens in 94.5% of patients. The validity of up 2 recombinant allergens has been confirmed for house dust mites. Systems of

expression allowing glycosylation of recombinant proteins were necessary to validate group 1 recombinant allergen proteins. Recombinant allergen Blo t 5 has been tested in vitro and in vivo, and was found to be effective in the detection of sensitization to Blomia tropicalis, a domestic allergen in subtropical countries. Only recombinant allergen Bla g 4 has been tested in vitro and in vivo, with positive reactions in almost 50% of subjects sensitized to crockroaches. Recombinant Asp f 1 was tested in subjects suffering from allergic bronchopulmonary aspergillosis, and was positive in 60 to 85% of cases. Studies are also available for recombinant allergens of phospholipase A2, the major allergen of bee venom. The consequences of the development of recombinant allergens are then analysed: better standardized allergens for diagnostic tests, study of the spectrum of specificities of the IgE induced by an allergen, quantification of specific IgE, better approach to cross-allergies using recombinant allergens of the main cross allergens. The application of recombinant allergens to basic research has led to production of modified recombinant allergens : synthetis of recombinant polypeptides corresponding to T epitopes, production of recombinant allergens isoforms with reduced allergenic activity, production of recombinant allergens of allergenic molecules modified by directed mutations. The use of these modified recombinant allergens is one line of research which, in the future, may lead to new modalities of specific desensitization. Other lines of research are also under investigation: inhibition of antigen-antibody reactions by the use of recombinant Fab-blocking molecules, and recombinant molecules: of immunodominant haptens.

L8 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3

1993:90720 Document No.: PREV199395045916. Expression and thrombin cleavage of Poa p IX recombinant allergens fused to glutathione-S-transferase. Olsen, Egil; Mohapatra, Shyam S. [Reprint author]. Dep. Immunology, Univ. Manitoba, 608-730 Williams Avenue, Winnipeg, Man. R3E OW3, Canada. International Archives of Allergy and Immunology, (1992) Vol. 98, No. 4, pp. 343-348.
CODEN: IAAIEG. ISSN: 1018-2438. Language: English.

AB The high-level expression and purification of Poa p IX recombinant grass pollen allergens were examined utilizing a modified pGEX plasmid, designated as pGEX 2T-1. This vector permits frame-1 ligation of lambda-gt11 cDNA inserts and cleavage of the recombinant allergenic protein from the fusion partner glutathione S-transferase. The expression of the fusion proteins in water-soluble form varied among the transformants of the same bacterial strain and also between different host strains. Purification of the fusion proteins by affinity chromatography employing glutathione agarose gel revealed that proteases in the bacterial lysate bound to the gel and were co-eluted with the fusion proteins. These proteases, which specifically degraded the recombinant proteins to varying degrees, were inhibited by both of the inhibitors, phenylmethylsulfonyl fluoride and aprotinin. Cleavage by thrombin of the fusion proteins indicated that the structure of the individual protein affected the thrombin accessibility to the cleavage site. Increased concentration of thrombin partly compensated this effect, but resulted in a broader specificity of the enzyme. By contrast, cleavage of the fusion protein when it was still attached to the glutathione gel was convenient and led to purification of the product devoid of proteolytic activity. Since almost all the recombinant allergens have been cloned in lambda-gt11 vector, the pGEX 2T-1 vector reported herein will facilitate the synthesis, purification of the corresponding allergenic proteins or their peptides in soluble and biologically active forms.

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L2             0 S L1 AND HEAT-KILLED
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L4       1162694 S ESCHERICHIA COLI
L5           462 S L4 AND RECOMBINANT ALLERGEN
L6            19 S L5 AND MODIFIED
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L12            1 L11 AND ALLERGEN

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L12  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
2004:1121739   Document No. 142:278913  Sensitization and allergic response and
      intervention therapy in animal models.  Helm, Ricki M.; Burks, A. Wesley
      (Department of Microbiology/Immunology, University of Arkansas for Medical
      Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA).  Journal of AOAC
      International, 87(6), 1441-1447 (English) 2004.  CODEN: JAINEE.  ISSN:
      1060-3271.  Publisher: AOAC International.
AB    A review is presented of 3 murine models and a swine neonatal model used
      to investigate immunotherapeutic options.  In Model 1, mutation of linear
      IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1
      is discussed with respect to expression in transgenic tobacco plants and
      correct folding following expression in the pET16b construct.  In Model 2,
      the mutations of Ara h 1 were assessed for use as an immunotherapeutic
      agent.  Although some protective benefit was observed with the modified Ara h
      1 protein, animals desensitized with heat-killed E.
      coli prepns. showed increased protection to challenge.  In Model
      3, soybean homologs to peanut proteins were investigated to determine if
      soybean immunotherapy can potentially provide benefit to peanut-allergic
      subjects.  Although some protection was provided, addnl. experimentation
      with respect to optimal doses for sensitization and challenge will need to
      be investigated.  In Model 4, the neonatal swine model was used to profile
      different foods (low to moderate to high sensitizing) similar to food
      allergies in humans.  Evidence suggests such feasibility; however,
      threshold levels for sensitization and allergic responses will need addnl.
      study.  In summary, murine and swine animal models are being used to
      address immunotherapeutic avenues and investigation into the mechanisms of
      food-allergic sensitization.

=> s heat-killed E coli
L13          230 HEAT-KILLED E COLI

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L14           15 L13 AND VACCINE

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L15 9 DUP REMOVE L14 (6 DUPLICATES REMOVED)

=> d l15 1-9 cbib abs

L15 ANSWER 1 OF 9 MEDLINE on STN
2004340100. PubMed ID: 15189567. NF-kappaB p50 facilitates neutrophil accumulation during LPS-induced pulmonary inflammation. Mizgerd Joseph P; Lupa Michal M; Spieker Matt S. (Physiology Program, Harvard School of Public Health, Boston, MA, 02115 USA.. jmizgerd@hsph.harvard.edu) . BMC immunology [electronic resource], (2004 Jun 9) Vol. 5, pp. 10. Electronic Publication: 2004-06-09. Journal code: 100966980. E-ISSN: 1471-2172. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Transcription factors have distinct functions in regulating immune responses. During Escherichia coli pneumonia, deficiency of NF-kappaB p50 increases gene expression and neutrophil recruitment, suggesting that p50 normally limits these innate immune responses. p50-deficient mice were used to determine how p50 regulates responses to a simpler, non-viable bacterial stimulus in the lungs, E. coli lipopolysaccharide (LPS). RESULTS: In contrast to previous results with living E. coli, neutrophil accumulation elicited by E. coli LPS in the lungs was decreased by p50 deficiency, to approximately 30% of wild type levels. Heat-killed E. coli induced neutrophil accumulation which was not decreased by p50 deficiency, demonstrating that bacterial growth and metabolism were not responsible for the different responses to bacteria and LPS. p50 deficiency increased the LPS-induced expression of kappaB-regulated genes essential to neutrophil recruitment, including KC, MIP-2, ICAM-1, and TNF-alpha suggesting that p50 normally limited this gene expression and that decreased neutrophil recruitment did not result from insufficient expression of these genes. Neutrophils were responsive to the chemokine KC in the peripheral blood of p50-deficient mice with or without LPS-induced pulmonary inflammation. Interleukin-6 (IL-6), previously demonstrated to decrease LPS-induced neutrophil recruitment in the lungs, was increased by p50 deficiency, but LPS-induced neutrophil recruitment was decreased by p50 deficiency even in IL-6 deficient mice. CONCLUSION: p50 makes essential contributions to neutrophil accumulation elicited by LPS in the lungs. This p50-dependent pathway for neutrophil accumulation can be overcome by bacterial products other than LPS and does not require IL-6.

L15 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1
96101747. PubMed ID: 7494229. Induction of T-cell immunity against Ras oncoproteins by soluble protein or Ras-expressing Escherichia coli. Fenton R G; Keller C J; Hanna N; Taub D D. (Division of Clinical Sciences, National Cancer Institute, National Cancer Institute-Frederick Cancer Research and Development Center (NCI-FCRDC), MD 21702, USA.) Journal of the National Cancer Institute, (1995 Dec 20) Vol. 87, No. 24, pp. 1853-61. Journal code: 7503089. ISSN: 0027-8874. Pub. country: United States. Language: English.

AB BACKGROUND: Point mutations in the ras proto-oncogene that activate its oncogenic potential occur in approximately 30% of human cancers. Previous studies have demonstrated that T-cell immunity against some forms of mutant Ras proteins could be elicited, and some effectiveness against tumors expressing activated Ras has been reported. PURPOSE: The goal of this study was to determine if immunization of mice with two forms of mutant Ras protein can induce high levels of Ras mutation-specific T-cell immunity in vitro and tumor regression in vivo. METHODS: Mice (BALB/c or C3H/HeJ) were immunized subcutaneously at 2-week intervals with purified Ras oncoproteins mixed with the immunologic adjuvants Antigen Formulation or QS-21, both of which have been shown to enhance the induction of T-cell-mediated immunity when included as components of soluble protein vaccines. In some experiments, mice were immunized directly with heat-killed Escherichia coli that had been induced to express one of the mutant Ras proteins. Spleen cells plus lymph node cells from Ras-immunized mice were tested in vitro for lysis of syngeneic

Ras-expressing tumor cells and proliferation in response to mutant Ras peptides. For some of the cytolytic activity experiments, the spleen cells were grown under TH1 conditions (growth in presence of interleukin 2, interferon gamma, and an antibody directed against interleukin 4 to stimulate a cell-mediated immune response) or TH2 conditions (growth in presence of interleukins 2 and 4 to stimulate a humoral immune response). The specificity of immunity was examined in vivo by challenge of Ras-immunized mice with syngeneic tumor cells expressing mutant Ras oncoproteins (HaBalb, i.e., BALB/c mouse cells expressing Ras with arginine substituted at amino acid position 12 [Arg 12 Ras]; C3HL61, i.e., C3H/HeJ mouse cells expressing Ras with leucine substituted at position 61 [Leu 61 Ras]). Ten mice per group were used in each experiment. RESULTS: Proliferative and cytolytic T-cell responses directed against the Arg 12 Ras protein were generated in BALB/c mice, resulting in protection against challenge with cells expressing Arg 12 Ras and therapeutic benefit in mice bearing established tumors expressing this protein. In C3H/HeJ mice, high levels of cytolytic and proliferative responses were induced against Leu 61 Ras. Immunization with heat-killed E.
coli genetically engineered to express Leu 61 Ras also led to the induction of anti-Ras T-cell immunity. T cells grown under TH1 conditions were cytolytic against Ras-transformed tumor cells, whereas those grown under TH2 conditions were not. CONCLUSIONS: Immunization as described here leads to Ras mutation-specific antitumor immunity in vitro and in vivo, with therapeutic efficacy in an established tumor model.

L15 ANSWER 3 OF 9 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
1985:233270 Document No.: PREV198579013266; BA79:13266. IMMUNOCONGLUTININ LEVELS IN CHICKS VACCINATED WITH SALMONELLA-GALLINARUM 9R SALMONELLA-PULLORUM E-79 OR ESCHERICHIA-COLI 020 VACCINES AND EXPERIMENTALLY INFECTED WITH SALMONELLA-GALLINARUM. JAISWAL T N [Reprint author]; MITTAL K R. COLLEGE OF VET SCIENCE AND ANIMAL HUSBANDRY, GUJARAT AGRIC UNIVERSITY, SK NAGAR, DANTIWADA, BANASKANTHA-395 506. Indian Veterinary Medical Journal, (1984) Vol. 8, No. 1, pp. 9-13.
CODEN: IVMJDL. ISSN: 0250-5266. Language: ENGLISH.
AB Vaccination of chicks with live S. gallinarum (9R) vaccines with or without adjuvant caused an initial fall in the levels of pre-existing autostimulated immunoconglutinin (IK) by the 10th day but a slow increase in the IK level by 21st day postvaccination. Heat-killed S. pollorum (E79) and heat-killed E. coli
(020) vaccines caused no such reduction in the IK level during the post-vaccination period. An increase in the IK level during post-vaccination period in these groups of chickens were observed. Challenge infection with S. gallinarium (V) in all the vaccinated groups of birds showed a marked decrease in IK level during the early challenge period indicating the involvement of IK in the host parasite reaction. The IK level increased by the 21st day post-challenge. Evidently, involvement of IK may help in host defense only in initial stages but eventually fail to protect chicks against S. gallinarum infection when the causative agent manages to enter the cells when both specific antibodies and nonspecific serum factor like IK fail to be effective.

L15 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 2
82190380. PubMed ID: 7042755. Induction of immunity against lethal Haemophilus influenzae type b infection by Escherichia coli core lipopolysaccharide. Marks M I; Ziegler E J; Douglas H; Corbeil L B; Braude A I. The Journal of clinical investigation, (1982 Apr) Vol. 69, No. 4, pp. 742-9. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB Efforts to prevent Haemophilus influenzae type b (HIB) infections in infancy have been hampered by the low immunogenicity of capsular polysaccharide vaccines in children younger than 18 mos. In searching for alternate immunogens, we have studied the protective potential of polysaccharide-poor, lipid-rich endotoxin (LPS) core in experimental HIB infections. Because all gram-negative bacteria have similar LPS core structures, we were able to use as vaccine the

J5 mutant of Escherichia coli 0111, the LPS of which consists only of core components, and thus to avoid problems in interpretation arising from vaccine contamination with non-LPS HIB immunogens. Mice were given graded inocula of HIB and developed lethal infection analogous to human HIB disease when virulence was enhanced with mucin and hemoglobin. After active immunization with heat-killed E. coli J5, 40/50 (80%) of infected mice survived, compared with 14/50 (28%) of saline-immunized controls (P less than 0.005). Passive immunization with rabbit antiserum against E. coli J5 prevented lethal HIB infection when administered 24 or 72 h before or 3 h after infection. This protection was abolished by adsorption of antiserum with purified J5 LPS, with survival reduced from 14/24 to 0/24 (P less than 0.005). Furthermore, rabbit antiserum to purified J5 LPS gave just as potent protection against death as antiserum to whole J5 cells. These studies demonstrate that immunity to core LPS confers protection against experimental murine HIB infection and provide the framework for a new approach to prevention of human disease from HIB.

L15 ANSWER 5 OF 9 MEDLINE on STN

81281536. PubMed ID: 7023456. Consequences of active or passive immunization of turkeys against Escherichia coli O78. Arp L H. Avian diseases, (1980 Oct-Dec) Vol. 24, No. 4, pp. 808-15. Journal code: 0370617. ISSN: 0005-2086. Pub. country: United States. Language: English.

AB Turkeys were injected at 7 and 14 days of age with live, heat-killed or formalin-killed Escherichia coli O78. Other turkeys were passively immunized at 22 days of age with hyperimmune serum produced against live or heat-killed E. coli O78. All turkeys were challenged at 24 days of age with E. coli O78. Turkeys immunized intramuscularly or intratracheally with live E. coli O78 were protected from death, whereas few turkeys given killed E. coli O78 were protected. Passively immunized turkeys were protected from death regardless of whether live or heat-killed E. coli O78 was used to produce the hyperimmune serum. Most turkeys that survived challenge developed septic polysynovitis 2--4 days after challenge.

L15 ANSWER 6 OF 9 MEDLINE on STN

76189304. PubMed ID: 818014. Antiviral activity of Brucella abortus preparations; separation of active components. Feingold D S; Keleti G; Youngner J S. Infection and immunity, (1976 Mar) Vol. 13, No. 3, pp. 763-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Injection into mice of heat-killed Brucella abortus or aqueous ether-extracted B. abortus (Bru-pel) induced a "virus-type" interferon response, with peak titers at 6.5 h. The animals also were protected against challenge with otherwise lethal doses of Semliki forest virus. Extraction of either heated B. abortus or BRU-PEL with a mixture of chloroform-methanol (2:1, vol/vol) (C-M(yielded an insoluble residue (extracted cells) and a C-M extract. Neither extracted cells nor C-M extract alone induced interferon or afforded protection against Semliki forest virus infection in mice. Full interferon-inducing and protective activity was restored when extracted cells were recombined with C-M extract. C-M extract from heat-killed Escherichia coli also was effective in restoring activity to extracted Brucella cells. Neither heat-killed E. coli nor its C-M extract was active, nor was C-M estracted E. coli recombined with the C-M extract from B. abortus. These results suggest that the interferon-inducing and antiviral protective properties of B. abortus are constituted of a C-M-extractable component that is common to B. abortus and E. coli and an unextractable component that is unique to B. abortus.

L15 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2007 ACS on STN

1974:567727 Document No. 81:167727 Intestinal antibody secretion in the young pig in response to oral immunization with Escherichia coli. Porter, P.; Kenworthy, R.; Noakes, D. E.; Allen, W. D. (Unilever Res.,

Sharnbrook/Bedford, UK). Immunology, 27(5), 841-53 (English) 1974. CODEN: IMMUAM. ISSN: 0019-2805.

AB Intestinal immunoglobulins and antibodies in the local immune response to E. coli O somatic antigens was studied in young fistulated pigs. Antibody levels in intestinal secretion were raised for .apprx.2-3 weeks following a single local antigenic challenge with a heat-killed aqueous suspension of E. coli. A 2nd challenge provoked a similar response suggesting a lack of immunol. memory. Antibody activity in the secretions was predominantly associated with IgA and immunofluorescent studies of biopsy specimens from these pigs indicated that intestinal synthesis and secretion of IgA had begun by the 10th day of life. Studies of piglets reared with the sow indicated that oral immunization with E. coli antigen after 10 days of age stimulated intestinal antibody secretion before weaning at 3 weeks. The response of gnotobiotic pigs to oral immunization and infection was evaluated by immunofluorescent histol. of the intestinal mucosa. Repeated oral administration of heat-killed E. coli O8 gave an immunocyte response in the lamina propria numerically comparable with that produced by infection. The early response was dominated by cells of the IgM class whereas after 3 weeks IgA cells predominated. In the germ-free animal very few immunoglobulin-containing cells were detected. In vitro studies of antibacterial activity indicated that the most probable mechanism of immunol. control in the alimentary tract is bacteriostasis.

L15 ANSWER 8 OF 9 EMBASE

75027648 EMBASE Document No.: 1975027648. The effect of active immunisation on ascending pyelonephritis in the rat. Radford N.J.; Chick S.; Ling R.; et al.. KRUF Inst. Ren. Dis., Welsh Nat. Sch. Med., Roy. Infirm., Cardiff, United Kingdom. J.PATH. Vol. 112, No. 3, pp. 169-175 1974. CODEN: JPBAA7
Language: English.

AB In the rat, active immunization with heat killed E. coli serotype 078 vaccine produced a high titer of IgM anti O antibody after 14 days. At this time, lower titers of IgG anti O antibodies were found in some of the animals. These antibodies did not prevent bacterial invasion of the kidney nor did they affect the incidence or severity of the renal scarring following ascending infection with E. coli serotype 078. Fourteen days after immunization with a formalin killed vaccine very high titers of IgM and IgG anti K antibodies were noted; these were in excess of 1 in 5120. It was shown that these antibodies reduced the severity but not the frequency of renal scarring following ascending E. coli infection.

L15 ANSWER 9 OF 9 MEDLINE on STN

71078403. PubMed ID: 4923787. [Oral immunization against coli enteritis with streptomycin-dependent E. coli. V. Different efficiency of live Sm-d and heat killed E. coli O111 B4 vaccine in settling of the homologous Sm-r strain in mice with antibiotic sterilized intestine]. Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Coli-Keimen. V. Unterschiedliche Wirksamkeit von Impfstoffen aus lebenden Streptomycin-dependenten und hitzeabgetoteten EC-O111 B4-Bakterien auf die Hemmung der Ansiedlung des homologen Streptomycin-resistenten Stammes bei darmsterilen Mausen. Lindek; Koch H. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale, (1970) Vol. 215, No. 3, pp. 286-95. Journal code: 0337744. ISSN: 0372-8110. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: German.

=> s l11 and allergen
L16 1 L11 AND ALLERGEN

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L16 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN
2004:1121739 Document No. 142:278913 Sensitization and allergic response and intervention therapy in animal models. Helm, Ricki M.; Burks, A. Wesley (Department of Microbiology/Immunology, University of Arkansas for Medical Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA). Journal of AOAC International, 87(6), 1441-1447 (English) 2004. CODEN: JAINEE. ISSN: 1060-3271. Publisher: AOAC International.
AB A review is presented of 3 murine models and a swine neonatal model used to investigate immunotherapeutic options. In Model 1, mutation of linear IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1 is discussed with respect to expression in transgenic tobacco plants and correct folding following expression in the pET16b construct. In Model 2, the mutations of Ara h 1 were assessed for use as an immunotherapeutic agent. Although some protective benefit was observed with the modified Ara h 1 protein, animals desensitized with heat-killed E. coli prepns. showed increased protection to challenge. In Model 3, soybean homologs to peanut proteins were investigated to determine if soybean immunotherapy can potentially provide benefit to peanut-allergic subjects. Although some protection was provided, addnl. experimentation with respect to optimal doses for sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

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L18 2 RECOMBINANT MODIFIED ALLERGEN

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L19 ANSWER 1 OF 1 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 1
2004135126 EMBASE [Hymenoptera venom allergy: Recent developments and perspectives in diagnosis and immunotherapy]. ALLERGIES AUX VENINS D'HYMENOPTERES: PERSPECTIVES DE PROGRES DANS LE DIAGNOSTIC ET LE TRAITEMENT D'HYPOSENSIBILISATION. Muller U.R.. U.R. Muller, Medizinische Klinik, Spital Bern Ziegler, Morillonstrasse 75-91, Bern CH-3006, Bern, Switzerland. ulrich.mueller@spitalbern.ch. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 44, No. 3, pp. 281-285 2004.
Refs: 34.
ISSN: 0335-7457. CODEN: RFAIBB
S 0335-7457(04)00017-6. Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20040412. Last Updated on STN: 20040412
AB There is a considerable potential to improve both diagnosis and immunotherapy in patients allergic to hymenoptera venoms. Among available diagnostic procedures, the basophil activation test appears interesting owing to its high specificity and sensitivity. This test is, however, expensive and without predictive value with regard to the protection induced by immunotherapy. Estimation of IL10 in lymphocyte cultures stimulated with the allergen may be more informative in this situation. An elevated basal serum tryptase level is a risk factor for particularly

severe anaphylactic reactions and hence an indication for prolonged immunotherapy. Diagnostic tests with a cocktail of the major recombinant venom allergens have superior specificity compared to tests with the whole venom. Recombinant modified allergens or T
cell epitope peptides no longer react with B cell epitopes of specific IgE while their reactivity with T cell epitopes is conserved. They will induce fewer side effects but they still be effective for immunotherapy. A reduction of side effects during the initial phase of immunotherapy can also be achieved by pre-medication with antihistamines. .COPYRGT. 2003 Elsevier SAS. Tous droits reserves.

=> s modified allergen
L20 557 MODIFIED ALLERGEN

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L21 0 L20 AND PEAUNT

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L23 ANSWER 1 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
2006:1035827 The Genuine Article (R) Number: 094PN. Immunological mechanisms of allergen-specific immunotherapy. Larche M (Reprint); Akdis C A; Valenta R. McMaster Univ, Dept Med, Div Clin Immunol & Allergy, 1200 Main St W, Hamilton, ON L8N 3Z5, Canada (Reprint); McMaster Univ, Dept Med, Div Clin Immunol & Allergy, Hamilton, ON L8N 3Z5, Canada; Univ London Imperial Coll Sci Technol & Med, MRC & Asthma UK Ctr Allerg Mechanisms Asthma, Dept Allergy & Clin Immunol, Natl Heart & Lung Inst, Fac Med, London SW7 2AZ, England; Swiss Inst Allergy & Asthma Res, CH-7270 Davos, Switzerland; Med Univ Vienna, Div Immunopathol, Dept Pathophysiol, Ctr Physiol & Pathophysiol, A-1090 Vienna, Austria. m.larche@imperial.ac.uk. NATURE REVIEWS IMMUNOLOGY (OCT 2006) Vol. 6, No. 10, pp. 761-771. ISSN: 1474-1733 . Publisher: NATURE PUBLISHING GROUP, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Allergen-specific immunotherapy has been carried out for almost a century and remains one of the few antigen-specific treatments for inflammatory diseases. The mechanisms by which allergen-specific immunotherapy exerts its effects include the modulation of both T-cell and B-cell responses to allergen. There is a strong rationale for improving the efficacy of allergen-specific immunotherapy by reducing the incidence and severity of adverse reactions mediated by IgE. Approaches to address this problem include the use of modified allergens,
novel adjuvants and alternative routes of administration. This article reviews the development of allergen-specific immunotherapy, our current understanding of its mechanisms of action and its future prospects.

L23 ANSWER 2 OF 11 MEDLINE on STN DUPLICATE 1
2005530273. PubMed ID: 16189800. Allergenic characteristics of a modified peanut allergen. King Nina; Helm Ricki; Stanley J Steven; Vieths Stefan; Luttkopf Dirk; Hatahet Lina; Sampson Hugh; Pons Laurent; Burks Wesley; Bannon Gary A. (Department of Biochemistry & Molecular Biology, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, USA.) Molecular nutrition & food research,

(2005 Oct) Vol. 49, No. 10, pp. 963-71. Journal code: 101231818. ISSN: 1613-4125. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Attempts to treat peanut allergy using traditional methods of allergen desensitization are accompanied by a high risk of anaphylaxis. The aim of this study was to determine if modifications to the IgE-binding epitopes of a major peanut allergen would result in a safer immunotherapeutic agent for the treatment of peanut-allergic patients. IgE-binding epitopes on the Ara h 2 allergen were modified, and modified Ara h 2 (mAra h 2) protein was produced. Wild-type (wAra h 2) and mAra h 2 proteins were analyzed for their ability to interact with T-cells, their ability to bind IgE, and their ability to release mediators from a passively sensitized RBL-2H3 cell line. Multiple T-cell epitopes were identified on the major peanut allergen, Ara h 2. Ara h 2 amino acid regions 11-35, 86-125, and 121-155 contained the majority of peptides that interact with T-cells from most patients. The wAra h 2 and mAra h 2 proteins stimulated proliferation of T-cells from peanut -allergic patients to similar levels. In contrast, the mAra h 2 protein exhibited greatly reduced IgE-binding capacity compared to the wild-type allergen. In addition, the modified allergen released significantly lower amounts of beta-hexosaminidase, a marker for IgE-mediated RBL-2H3 degranulation, compared to the wild-type allergen.

L23 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN

2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L23 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 2
2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L23 ANSWER 5 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
2003:28190 The Genuine Article (R) Number: 625KX. Clinical aspects of food allergy. Papageorgiou P S (Reprint). 58 Voutsina St, Holargos 15561, Greece (Reprint); Univ Athens, Sch Med, P&A Kyriakou Childrens Hosp, Allergy Unit, GR-11527 Athens, Greece. BIOCHEMICAL SOCIETY TRANSACTIONS (NOV 2002) Vol. 30, Part 6, pp. 901-906. ISSN: 0300-5127. Publisher: PORTLAND PRESS, 59 PORTLAND PLACE, LONDON W1N 3AJ, ENGLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Food allergy affects 2.5 % of adults and 6-8 % of children, and is a leading cause of life-threatening anaphylactic episodes. Food allergy is defined as an adverse reaction to foods that is mediated immunologically and involves specific IgE or non-IgE mechanisms. In this review only IgE-related food allergy will be considered. Many food allergens are glycoproteins, but they do not share any striking biochemical similarities. The definition of many food proteins at the molecular level has tremendously facilitated our understanding of clinical syndromes and seemingly bizarre observations. Clinical manifestations of food allergy include symptoms of the gastrointestinal, cutaneous and respiratory systems, as well as systemic anaphylaxis. The diagnosis of food allergy involves a stepwise approach, including medical history taking, demonstration of specific IgE and confirmation by oral food challenge. The management of the food-allergic patient at present consists of avoidance of the culprit food and education, while future advances may

include specific immunotherapy with modified allergens or DNA vaccination.

L23 ANSWER 6 OF 11 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 3

2002:301398 Document No.: PREV200200301398. Immunotherapy for peanut allergy using modified allergens and a bacterial adjuvant. Stanley, Joseph Steve [Reprint author]; Buzen, Fred [Reprint author]; Cockrell, Gael [Reprint author]; West, Mike [Reprint author]; Srivastava, Kamal D.; Li, X. M.; Sampson, Hugh A.; Burks, Wesley [Reprint author]; Bannon, Gary A. [Reprint author]. University of Arkansas, Little Rock, AR, USA. Journal of Allergy and Clinical Immunology, (January, 2002) Vol. 109, No. 1 Supplement, pp. S93. print.
Meeting Info.: 58th Annual Meeting of the American Academy of Allergy, Asthma and Immunology. New York, NY, USA. March 01-06, 2002. American Academy of Allergy, Asthma, and Immunology.
CODEN: JACIBY. ISSN: 0091-6749. Language: English.

L23 ANSWER 7 OF 11 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2002:530650 The Genuine Article (R) Number: 563MD. Modification of peanut allergen Ara h 3: Effects on IgE binding and T cell stimulation. Rabjohn P; West C M; Connaughton C; Sampson H A; Helm R M (Reprint); Burks A W; Bannon G A. Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Slot 512, 1120 Marshall St, Little Rock, AR 72202 USA (Reprint); Univ Arkansas Med Sci, ACHRI, Dept Biochem & Mol Biol, Little Rock, AR 72202 USA; Univ Arkansas Med Sci, ACHRI, Dept Pediat, Little Rock, AR 72202 USA; Mt Sinai Sch Med, Dept Pediat, New York, NY USA. INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (MAY 2002) Vol. 128, No. 1, pp. 15-23. ISSN: 1018-2438. Publisher: KARGER, ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Peanut allergy is a major health concern due to the increased prevalence, potential severity, and chronicity of the reaction. The cDNA encoding a third peanut allergen, Ara h 3, has been previously cloned and characterized. Mutational analysis of the Ara h 3 IgE-binding epitopes with synthetic peptides revealed that single amino acid changes at critical residues could diminish IgE binding. Methods: Specific oligonucleotides were used in polymerase chain reactions to modify the cDNA encoding Ara h 3 at critical IgE binding sites. Four point mutations were introduced into the Ara h 3 cDNA at codons encoding critical amino acids in epitopes 1, 2, 3 and 4. Recombinant modified proteins were used in SDS-PAGE/Western IgE immunoblot, SDS-PAGE/Western IgE immunoblot inhibition and T cell proliferation assays to determine the effects of these changes on in vitro clinical indicators of peanut hypersensitivity. Results: Higher amounts of modified Ara h 3 were required to compete with the wild-type allergen for peanut -specific serum IgE. Immunoblot analysis with individual serum IgE from Ara-h-3-allergic patients showed that IgE binding to the modified protein decreased similar to35-85% in comparison to IgE binding to wildtype Ara h 3. Also, the modified Ara h 3 retained the ability to stimulate T cell activation in PBMCs donated by Ara-h-3-allergic patients. Conclusions: The engineered hypoallergenic Ara h 3 variant displays two characteristics essential for recombinant allergen immunotherapy; it has a reduced binding capacity for serum IgE from peanut-hypersensitive patients and it can stimulate T-cell proliferation and activation. Copyright (C) 2002 S, Karger AG, Basel.

L23 ANSWER 8 OF 11 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 4

2001120857 EMBASE [Peanut allergy]. ALLERGIE A L'ARACHIDE. Dutau G.; Rance F.. G. Dutau, Unite des maladies respiratoires, Hopital des Enfants, 330, avenue de Grande-Bretagne, 31026 Toulouse Cedex 3, France. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 41, No. 2, pp. 187-198 2001.

Refs: 98.
ISSN: 0335-7457. CODEN: RFAIBB
Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20010412. Last Updated on STN: 20010412

AB Peanut allergy, which is frequent in the United States and was much less so in Europe up to the mid-eighties, has become a major problem in many industrialized countries. Peanut consumption is high in Eastern Europe, the United Kingdom, The Netherlands, Germany and France. The frequency of peanut allergy is between 0.5 and 0.7% in the general population. Two million Americans are now thought to be affected. In France peanuts are one of the most frequent allergens, lying second (27.4 %) to egg in food allergies in children, and holding first place in food allergies in children aged over 3 years. Sensitization occurs through ingestion, contact even if indirect, and inhalation. The symptoms, which affect the skin and the respiratory or gastrointestinal tract, appear a few minutes to a few hours after exposure. Serious reactions (anaphylactic shock, life-threatening reactions, sudden death) have been described. Asthma has a significantly higher association with peanut allergy than with other allergies, taken overall. As with other food allergies, diagnosis is based on history, prick-tests, screening for specific serum IgE and food challenge whose modalities (labial and oral challenge) are debated. For the time being, elimination is the only form of treatment. The development of a modified allergen as immunogenic as possible but practically without allergenic effects should give immunotherapy new impetus. Patients with severe peanut allergy should carry a card or wear a distinctive bracelet indicating their condition as well as an emergency kit including in particular epinephrine. .COPYRGT. 2001 Editions scientifiques et medicales Elsevier SAS.

L23 ANSWER 9 OF 11 MEDLINE on STN DUPLICATE 5

2001262411. PubMed ID: 11306930. Engineering, characterization and in vitro efficacy of the major peanut allergens for use in immunotherapy. Bannon G A; Cockrell G; Connaughton C; West C M; Helm R; Stanley J S; King N; Rabjohn P; Sampson H A; Burks A W. (Department of Biochemistry and Molecular Biology, Arkansas Children's Hospital Research Institute, Little Rock 72205, USA.. bannongarya@exchnage.uams.edu) . International archives of allergy and immunology, (2001 Jan-Mar) Vol. 124, No. 1-3, pp. 70-2. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Numerous strategies have been proposed for the treatment of peanut allergies, but despite the steady advancement in our understanding of atopic immune responses and the increasing number of deaths each year from peanut anaphylaxis, there is still no safe, effective, specific therapy for the peanut-sensitive individual. Immunotherapy would be safer and more effective if the allergens could be altered to reduce their ability to initiate an allergic reaction without altering their ability to desensitize the allergic patient. METHODS: The cDNA clones for three major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, have been cloned and characterized. The IgE-binding epitopes of each of these allergens have been determined and amino acids critical to each epitope identified. Site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, provided the reagents necessary to test our hypothesis that hypoallergenic proteins are effective immunotherapeutic reagents for treating peanut-sensitive patients. Modified peanut allergens were subjected to immunoblot analysis using peanut-positive patient sera IgE, T cell proliferation assays, and tested in a murine model of peanut anaphylaxis. RESULTS: In general, the modified allergens were poor competitors for binding of peanut-specific IgE when compared to their wild-type counterpart. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens. In addition, while there was

considerable variability between patients, the modified allergens retained the ability to stimulate T cell proliferation. CONCLUSIONS: These modified allergen genes and proteins should provide a safe immunotherapeutic agent for the treatment of peanut allergy.
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L23 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN
1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.
AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by masking the site with a compound that prevents IgE binding or by altering as little as a single amino acid within the protein, most typically a hydrophobic residue towards the center of the IgE-binding epitope, to eliminate IgE binding. The method allows the protein to be altered as minimally as possible, other than within the IgE-binding sites, while retaining the ability of the protein to activate T cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The examples use peanut allergens to demonstrate alteration of IgE binding sites. The critical amino acids within each of the IgE binding epitopes of the peanut protein that are important to Ig binding have been determined Substitution of even a single amino acid within each of the epitopes led to loss of IgE binding. Although the epitopes shared no common amino acid sequence motif, the hydrophobic residues located in the center of the epitope appeared to be most critical to IgE binding.

L23 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2007 ACS on STN
1983:556476 Document No. 99:156476 Some immunochemical studies of native and modified allergens. King, Te Piao; Giallongo, Agata (Rockefeller Univ., New York, NY, 10021, USA). Skandia International Symposia, Volume Date 1982, 15th(Theor. Clin. Aspects Allerg. Dis.), 215-36 (English) 1983. CODEN: SISYDD. ISSN: 0346-9069.
AB Studies were performed to induce significant suppression of specific IgE with very low doses of a highly immunogenic material (modified antigens). Two allergen-lectin conjugates were prepared; ragweed antigen E was conjugated with either peanut agglutinin or wheat germ agglutinin. These modified allergens were not more effective than the native allergen in suppressing specific IgE production

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L26 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN

2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L26 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

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L26 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.
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L28 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN
2003:855391 Document No. 139:363577 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael J.; Sosin, Howard B.; Sampson, Hugh; Bannon, Gary A.; Burks, A. Wesley; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.;

Maleki, Soheila J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (USA). U.S. Pat. Appl. Publ. US 2003202980 A1 20031030, 194 pp., Cont.-in-part of U.S. Ser. No. 494,096. (English). CODEN: USXXCO. APPLICATION: US 2002-100303 20020318. PRIORITY: US 1995-9455P 19951229; US 1996-717933 19960923; US 1998-73283P 19980131; US 1998-74633P 19980213; US 1998-74624P 19980213; US 1998-74590P 19980213; US 1998-106872 19980629; US 1998-141220 19980827; US 1998-191593 19981113; US 1999-241101 19990129; US 1999-240557 19990129; US 1999-248674 19990211; US 1999-248673 19990211; US 1999-122560P 19990302; US 1999-122565P 19990302; US 1999-122566P 19990302; US 1999-122450P 19990302; US 1999-122452P 19990302; US 1999-267719 19990311; US 2000-494096 20000128.

AB It has been determined that allergens, which are characterized by both humoral (IgE) and cellular (T-cell) binding sites, can be modified to be less allergenic by modifying the IgE binding sites. The IgE binding sites can be converted to non-IgE binding sites by altering as little as a single amino acid within the protein, preferably a hydrophobic residue towards the center of the IgE epitope, to eliminate IgE binding. Addnl. or alternatively a modified allergen with reduced IgE binding may be prepared by disrupting one or more of the disulfide bonds that are present in the natural allergen. The disulfide bonds may be disrupted chemical, e.g., by reduction and alkylation or by mutating one or more cysteine residues present in the primary amino acid sequence of the natural allergen. In certain embodiments, modified allergens are prepared by both altering one or more linear IgE epitopes and disrupting one or more disulfide bonds of the natural allergen. In certain embodiments, the methods of the present invention allow allergens to be modified while retaining the ability of the protein to activate T-cells, and, in some embodiments by not significantly altering or decreasing IgG binding capacity. The immunotherapeutics can be prepared in transgenic plants or animals; and administered in injection, aerosol, sublingual or topical form. The immunotherapeutics can also be encoded in gene for gene therapy and delivered by injecting into muscle or skin to induce tolerance. The Examples provided herein use peanut allergens to illustrate applications of the invention.

L28 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 1

2003:906632 Correction of: 2002:736063 Document No. 139:349665 Correction of: 137:277814 Modified anaphylactic food allergens with reduced IgE-binding ability for decreasing clinical reaction to allergy. Caplan, Michael; Sosin, Howard; Sampson, Hugh; Bannon, Gary A.; Burks, Wesley A.; Cockrell, Gael; Compadre, Cesar M.; Connaughton, Cathie; Helm, Ricki M.; King, Nina E.; Kopper, Randall A.; Maleki, Sohelia J.; Rabjohn, Patrick A.; Shin, David S.; Stanley, J. Steven (Panacea Pharmaceuticals, USA; et al.). PCT Int. Appl. WO 2002074250 A2 20020926, 299 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US9108 20020318. PRIORITY: US 2001-276822P 20010316.

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L28 ANSWER 3 OF 6 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 2

2001120857 EMBASE [Peanut allergy]. ALLERGIE A L'ARACHIDE. Dutau G.; Rance F.. G. Dutau, Unite des maladies respiratoires, Hopital des Enfants, 330, avenue de Grande-Bretagne, 31026 Toulouse Cedex 3, France. Revue Francaise d'Allergologie et d'Immunologie Clinique Vol. 41, No. 2, pp. 187-198 2001.
Refs: 98.
ISSN: 0335-7457. CODEN: RFAIBB
Pub. Country: France. Language: French. Summary Language: English; French.
Entered STN: 20010412. Last Updated on STN: 20010412

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L28 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2007 ACS on STN

1999:495393 Document No. 131:143513 Methods and reagents for decreasing allergic reactions. Sosin, Howard; Bannon, Gary A.; Burks, A. Wesley, Jr.; Sampson, Hugh A. (University of Arkansas, USA; Mt. Sinai School of Medicine, University of New York). PCT Int. Appl. WO 9938978 A1 19990805, 46 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US2031 19990129. PRIORITY: US 1998-PV73283 19980131; US 1998-PV74590 19980213; US 1998-PV74624 19980213; US 1998-PV74633 19980213; US 1998-141220 19980827.

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L28 ANSWER 5 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1998:146495 The Genuine Article (R) Number: YY214. Tolerogenic activity of polyethylene glycol-conjugated lysozyme distinct from that of the native counterpart. Ito H O (Reprint); So T; Hirata M; Koga T; Ueda T; Imoto T. Kyushu Univ, Fac Dent, Dept Biochem, Fukuoka 81282, Japan (Reprint); Kyushu Univ, Grad Sch Pharmaceut Sci, Fukuoka 81282, Japan. IMMUNOLOGY (FEB 1998) Vol. 93, No. 2, pp. 200-207. ISSN: 0019-2805. Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Conjugation of proteins with polyethylene glycol (PEG) has been reported to make the proteins tolerogenic. Native proteins are also potentially tolerogenic when given without adjuvants. We compared immunotolerogenic activities between PEG-conjugated and native hen egg-white lysozyme (HEL). BALB/c mice were given consecutive weekly intraperitoneal administrations of PEG-conjugated HEL, unmodified HEL or phosphate-buffered saline (PBS), for 3 weeks, then challenged with HEL in Freund's complete adjuvant. The pretreatment with PEG-HEL tolerized both T-cell and humoral responses, while native HEL tolerized only the T-cell response. Immunoglobulin G1 (IgG1) antibody was already elevated in HEL-pretreated mice prior to challenge immunization, followed by suppressed IgG2a and IgG2b. but spared IgG1 production after the antigen challenge. whereas PEG-HEL-pretreated mice produced no antibody in all IgG subclasses prior and subsequent to the antigen-challenge. Production of interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) by lymphoid cells in response to HEL in vitro was markedly suppressed in both the antigen-pretreated groups. while suppression of IL-4 production was evident only in PEG-HEL-, not in HEL-pretreated animals. Involvement of suppressor cells in these tolerance states was found to be unlikely. and the immunological property of PEG-HEL differed from deaggregated HEL that was similar to the original HEL. These results suggest a unique immunotolerogenic activity of PEG-conjugated proteins to suppl css both T-helper type-1 (Th1)and Th2-type responses, the result bring extensive inhibition of all IgG subclass responses, while tolerance induction by unconjugated soluble proteins tends to be targeted on Th1-, but spares Th2-type responses.

L28 ANSWER 6 OF 6 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

1996:258810 The Genuine Article (R) Number: UC578. Reduced immunogenicity of monomethoxypolyethylene glycol-modified lysozyme for activation of T cells . So T (Reprint); Ito H O; Koga T; Ueda T; Imoto T. KYUSHU UNIV, GRAD SCH PHARMACEUT SCI, FUKUOKA 81282, JAPAN; KYUSHU UNIV, SCH DENT, DEPT BIOCHEM, FUKUOKA 81282, JAPAN. IMMUNOLOGY LETTERS (JAN 1996) Vol. 49, No. 1-2, pp. 91-97. ISSN: 0165-2478. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Chemical modification of proteins with monomethoxypolyethylene glycol (mPEG) will reduce the immunogenicity of proteins. In the present study,

we evaluated the effect of mPEG modification on the capacity of hen egg-white lysozyme (HEL) to stimulate T cells. Lymph node cells (LNCs) from mice immunized with HEL or with mPEG-HEL conjugate were cultured with these antigens, then we measured the proliferation and IL-2 production. mPEG-modification lowered the T cell-activating capacity of HEL, both in vitro and in vivo. Neither toxicity, nor antigen non-specific immunosuppressive capacity was observed with mPEG-HEL and unconjugated mPEG. Suppressor cells were unlikely to be generated in the mPEG-HEL-primed LNCs. We next examined the behavior of mPEG-HEL during antigen processing. The capacity of HEL and mPEG-HEL to be incorporated by live cells was much the same. However, the susceptibility to various proteases, including endosomal/lysosomal enzymes, was significantly decreased by mPEG modification. The increased resistance of mPEG-HEL to proteolytic degradation implied that the conjugate was poorly presented to T cells, This may be an important factor related to the low immunogenicity of mPEG modified proteins.

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=> s l20 and shrimp
L29           5 L20 AND SHRIMP

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PROCESSING COMPLETED FOR L29
L30           1 DUP REMOVE L29 (4 DUPLICATES REMOVED)

=> d l30 cbib abs
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L30 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1

2000290936. PubMed ID: 10828721. Modulation of allergen-specific immune responses to the major shrimp allergen, tropomyosin, by specific targeting to scavenger receptors on macrophages. Rajagopal D; Ganesh K A; Subba Rao P V. (Department of Biochemistry, Indian Institute of Science, Bangalore, India.) International archives of allergy and immunology, (2000 Apr) Vol. 121, No. 4, pp. 308-16. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Tropomyosin from shrimp is the major cross-reacting crustacean food allergen. Earlier studies have led to the purification and immunochemical characterization of the major IgE binding epitopes of the allergen. Maleylated proteins are known to be specifically targeted to scavenger receptors on macrophage. Since antigens processed and presented by macrophages are known to elicit Th1 type of responses and allergic responses are characterized by polarization towards Th2 phenotype, the possibility of modulation of allergen-specific immune responses by targeting of tropomyosin to macrophage via scavenger receptor was explored. METHODS: The IgG and IgE binding potential of the native maleylated form of tropomyosin was carried out by ELISA and immunoblot. The ability of the native and maleylated form of allergen to induce in vitro proliferation of splenocytes from BALB/C mice immunized with both forms of allergen was tested. The in vitro production of IL-4 and IFN-gamma by splenocytes from mice immunized with the two forms of allergen was determined from culture supernatants. The in vivo production of serum IgG1 and IgG2a antibodies following immunization with native and modified allergens was monitored by ELISA. RESULTS: The maleylated form of tropomyosin was found to have reduced antigenicity and allergenicity as compared to its native counterpart. The modified allergen was, however, found to elicit a cellular response similar to native tropomyosin in vitro. Analysis of the cytokine profiles showed a modulation from an IL-4-dominant, proallergic, Th2 phenotype to an IFN-gamma-dominant, antiallergic, Th1 phenotype that could also be correlated to a modulation in the in vivo antibody isotype. CONCLUSION: The results suggest the possible potential for modulating allergic responses in vivo by selective targeting to macrophages.

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     (FILE 'HOME' ENTERED AT 12:12:48 ON 09 JAN 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 12:13:14 ON
     09 JAN 2007
L1          8476 S RECOMBINANT ESCHERICHIA COLI
L2             0 S L1 AND HEAT-KILLED
L3             0 S L1 AND MODIFIED ALLERGEN
L4       1162694 S ESCHERICHIA COLI
L5           462 S L4 AND RECOMBINANT ALLERGEN
L6            19 S L5 AND MODIFIED
L7             0 S L6 AND HEAT-KILLED
L8             9 DUP REMOVE L6 (10 DUPLICATES REMOVED)
L9        387782 S E COLI
L10            0 S L9 AND MODIFIED ALLERGEN
L11         3911 S L9 AND KILLED
L12            1 S L11 AND ALLERGEN
L13          230 S HEAT-KILLED E COLI
L14           15 S L13 AND VACCINE
L15            9 DUP REMOVE L14 (6 DUPLICATES REMOVED)
L16            1 S L11 AND ALLERGEN
L17            0 S L11 AND PET16
L18            2 S RECOMBINANT MODIFIED ALLERGEN
L19            1 DUP REMOVE L18 (1 DUPLICATE REMOVED)
L20          557 S MODIFIED ALLERGEN
L21            0 S L20 AND PEAUNT
L22           21 S L20 AND PEANUT
L23           11 DUP REMOVE L22 (10 DUPLICATES REMOVED)
L24            0 S L23 AND E COLI
L25            4 S L20 AND MILK
L26            3 DUP REMOVE L25 (1 DUPLICATE REMOVED)
L27            8 S L20 AND EGG
L28            6 DUP REMOVE L27 (2 DUPLICATES REMOVED)
L29            5 S L20 AND SHRIMP
L30            1 DUP REMOVE L29 (4 DUPLICATES REMOVED)

=> s l13 and inclusion body
L31            0 L13 AND INCLUSION BODY

=> s l13 and allergen
L32            1 L13 AND ALLERGEN

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L32  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2007 ACS on STN
2004:1121739  Document No. 142:278913  Sensitization and allergic response and
     intervention therapy in animal models.  Helm, Ricki M.; Burks, A. Wesley
     (Department of Microbiology/Immunology, University of Arkansas for Medical
     Sciences/ACHRI/ACNC, Little Rock, AR, 72202-3591, USA).  Journal of AOAC
     International, 87(6), 1441-1447 (English) 2004.  CODEN: JAINEE.  ISSN:
     1060-3271.  Publisher: AOAC International.
AB   A review is presented of 3 murine models and a swine neonatal model used
     to investigate immunotherapeutic options.  In Model 1, mutation of linear
     IgE-binding epitopes of Ara h 1 for the preparation of a hypoallergenic Ara h 1
     is discussed with respect to expression in transgenic tobacco plants and
     correct folding following expression in the pET16b construct.  In Model 2,
     the mutations of Ara h 1 were assessed for use as an immunotherapeutic
     agent.  Although some protective benefit was observed with the modified Ara h
     1 protein, animals desensitized with heat-killed
     E. coli prepns. showed increased protection to
     challenge.  In Model 3, soybean homologs to peanut proteins were
     investigated to determine if soybean immunotherapy can potentially provide
     benefit to peanut-allergic subjects.  Although some protection was
     provided, addnl. experimentation with respect to optimal doses for
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sensitization and challenge will need to be investigated. In Model 4, the neonatal swine model was used to profile different foods (low to moderate to high sensitizing) similar to food allergies in humans. Evidence suggests such feasibility; however, threshold levels for sensitization and allergic responses will need addnl. study. In summary, murine and swine animal models are being used to address immunotherapeutic avenues and investigation into the mechanisms of food-allergic sensitization.

=> s l11 and formaldehyde
L33 42 L11 AND FORMALDEHYDE

=> s l33 and allergen
L34 0 L33 AND ALLERGEN

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PROCESSING COMPLETED FOR L33
L35 19 DUP REMOVE L33 (23 DUPLICATES REMOVED)

=> d l35 1-19 cbib abs

L35 ANSWER 1 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
2006390023 EMBASE Detection of Escherichia coli O157:H7 using chicken immunoglobulin Y. Sunwoo H.H.; Wang W.W.; Sim J.S.. H.H. Sunwoo, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada. hsunwoo@ualberta.ca. Immunology Letters Vol. 106, No. 2, pp. 191-193 15 Aug 2006.
Refs: 8.
ISSN: 0165-2478. CODEN: IMLED6
S 0165-2478(06)00132-5. Pub. Country: Netherlands. Language: English. Summary Language: English.
Entered STN: 20060831. Last Updated on STN: 20060831
AB A sandwich ELISA technique was examined to detect Escherichia coli O157:H7 using chicken anti-E. coli O157:H7 IgY as the capture-antibody and an anti-E. coli O157 mouse mAb conjugated with biotin as the detection antibody. The anti-E. coli O157:H7 IgY was harvested from eggs laid by hens (23 weeks of age, Single Comb White Leghorn) immunized with formalin-killed E. coli O157:H7. The IgY was purified by water dilution methods and gel chromatography on Sephacryl S-300 followed by ammonium sulfate precipitation. The sensitivity (CFU/ml) of sandwich ELISA for the E. coli O157:H7 was repeatedly examined with 10 replicates of each sample and a standard curve was plotted. The sandwich ELISA can detect as low as 40 CFU/ml of E. coli O157:H7. The data suggest that chicken IgY-based sandwich ELISA provides a reliable, inexpensive and sensitive assay for the detection of the food-borne pathogen E. coli O157:H7. .COPYRGT. 2006 Elsevier B.V. All rights reserved.

L35 ANSWER 2 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN
2001304745 EMBASE Evaluation of in vitro antibacterial activity of some disinfectants on Escherichia coli serotypes. El-Naggar M.Y.M.; Akeila M.A.; Turk H.A.; El-Ebady A.A.; Sahaly M.Z.. Dr. M.Y.M. El-Naggar, Botany/Microbiology Department, Faculty of Science, Alexandria University, Alexandria, Egypt. Moustafa64@Yahoo.com. Journal of General and Applied Microbiology Vol. 47, No. 2, pp. 63-73 2001.
Refs: 33.
ISSN: 0022-1260. CODEN: JGAMA
Pub. Country: Japan. Language: English. Summary Language: English.
Entered STN: 20010913. Last Updated on STN: 20010913
AB Three disinfectants commonly used in poultry farms (formalin, TH4+, and Virkon-S) were chosen for the present study. The effect of disinfectant concentration and the duration of exposure to these disinfectants on the

survival of Escherichia coli serotypes (O114:K-, O86, O55:K39, and O86:K60) were investigated. Formalin (0.6%), TH4+ (0.06%), and Virkon (0.5%) all killed the four serotypes within 5 min of exposure. As the disinfectant concentration decreases, the length of exposure time to kill serotype increases. At 0.03%, 0.007%, and 0.03% of formalin, TH4+ and Virkon-S concentrations failed to kill the four E. coli serotypes within 360 min, respectively. An improvement of the inhibitory effect of these disinfectants occurred when added together with the inoculum instead of an established population. The influence of formalin, TH4+, and Virkon-S on the cell morphology of E. coli O55:K39 was investigated by using transmission electron microscopy. Formalin-treated cells exhibited normal cell morphology, with the exception that the treated cell was less fimbriated, and more destruction of pili increased when formalin concentrations were doubled. Cells treated with TH4+ (0.03%) showed destruction of the cell wall and cell surface membrane after 5 min. Cell filamentation occurred at 0.015% and increased with the increase of exposure time to this drug. Spheroplasts were observed only when cells were treated with 0.125% Virkon-S for 60 min, and cell lysis started to occur when 0.25% Virkon-S was applied for 15 min. Scanning electron microscope study revealed that Virkon-S at 0.03% and TH4+ at 0.007% completely prevented the adherence of E. coli O55:K39 serotype to chicken tracheal organ, whereas formalin (0.03%) disinfection minimized the adherence of E . coli cells to tracheal explants after 360 min of incubation.

L35 ANSWER 3 OF 19 MEDLINE on STN DUPLICATE 1

2000137497. PubMed ID: 10675032. Identification and cloning of an aspartyl proteinase from Coccidioides immitis. Johnson S M; Kerekes K M; Zimmermann C R; Williams R H; Pappagianis D. (Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis 95616, USA.. smjohnson@ucdavis.edu) . Gene, (2000 Jan 11) Vol. 241, No. 2, pp. 213-22. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB A 45 kDa protein was isolated from a soluble vaccine prepared from formaldehyde-killed spherules of Coccidioides immitis. From the N-terminal amino acid sequence, the protein yielded a 17-amino-acid peptide that was homologous to sequences of other fungal aspartyl proteinases. The coccidioidal cDNA encoding the proteinase was amplified using oligonucleotide primers designed from the 45 kDa N-terminal amino acid sequence and a fungal aspartyl proteinase consensus amino acid sequence. The PCR product was cloned and sequenced, and the remaining 5' upstream and 3' downstream cDNA was amplified, cloned, and sequenced. The cDNA encoding the coccidioidal aspartyl proteinase open reading frame was cloned and the fusion protein containing a C-terminal His-tag expressed in E. coli. The recombinant aspartyl proteinase was purified by immobilized metal affinity chromatography. This recombinant protein will be used for further studies to evaluate its antigenicity, including protective immunogenicity.

L35 ANSWER 4 OF 19 MEDLINE on STN DUPLICATE 2

1998311328. PubMed ID: 9648994. Escherichia coli and Proteus mirabilis inhibit the perinuclear but not the circulating antineutrophil cytoplasmic antibody reaction. Yang P; Danielsson D; Jarnerot G. (Dept. of Medicine, Orebro Medical Centre Hospital, Sweden.) Scandinavian journal of gastroenterology, (1998 May) Vol. 33, No. 5, pp. 529-34. Journal code: 0060105. ISSN: 0036-5521. Pub. country: Norway. Language: English.

AB BACKGROUND: Perinuclear antineutrophil cytoplasmic antibodies (P-ANCA) are found in 48%-83% of serum samples from patients with ulcerative colitis (UC). Their pathogenic role and initiating stimuli are unknown. In contrast to patients with vasculitides and ANCA reactivities, the antibodies in UC patients do not react with myeloperoxidase (MPO) or proteinase 3 (PR3). The aim of the present study was to investigate whether bacterial species of the intestinal tract and other sources could interfere with P-ANCA in sera from patients with UC. METHODS: Seventeen P-ANCA-positive and anti-MPO-negative serum samples from patients with UC

were tested with Escherichia coli 014 and Staphylococcus aureus Wood 46. Six of these serum samples with different P-ANCA titres were selected to test further the influence of 15 different gram-negative or gram-positive bacterial strains. Six anti-MPO positive P-ANCA, 5 anti-PR3 positive C-ANCA, and 10 antinuclear antibody (ANA)-positive serum samples were used as controls. The antineutrophil cytoplasmic antibodies (ANCAs) were analysed by an indirect immunofluorescence method (IIF) on ethanol-fixed neutrophils, and the ANAs were tested by IIF on HEp-2 cells or rat liver tissues. The bacteria used in the experiments were either live or killed by formalin or glutaraldehyde fixation or heated at 80 degrees C for 30 min. The test was first performed as a bacterial absorption test with sedimented organisms and then at various temperatures with the supernatant from suspension of live bacteria. RESULTS: Both MPO-positive and MPO-negative P-ANCA reactivity was abolished by absorption of patient sera with live E. coli and Proteus mirabilis but not with bacteria representing members of 10 other species, suggesting that antibody reactivity was absorbed away. However, continued experiments indicated that the inhibition of P-ANCA was not due to classic antigen-antibody interactions but rather to decomposition of the antigenic substrate of the neutrophils by factors present in the supernatants of live E. coli and P. mirabilis. The activity of the supernatant was temperature-dependent, with strong activity at room temperature and 37 degrees C, no activity at 0 degrees C, and abolished by mild heat treatment (56 degrees or 60 degrees C). No activity was shown in the supernatants from bacteria treated with formaldehyde or glutaraldehyde. CONCLUSIONS: Soluble material from live E. coli and P. mirabilis has the capacity to decompose the antigenic substrate of neutrophils responsible for both MPO-positive and MPO-negative P-ANCA, most probably brought about through enzymatic activity. Anti PR3-positive C-ANCA were not affected, which suggests substrate specificity of the proposed enzymatic activity.

L35 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN

1998:303526 Document No. 129:107734 Endotoxin-effects of vaccination with Escherichia coli vaccines in the pig. Garcia, P.; Hakt, H.; Magnusson, U.; Kindahl, H. (Department of Obstetrics and Gynaecology and Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Swed.). Acta Veterinaria Scandinavica, 39(1), 135-140 (English) 1998. CODEN: AVSCA7. ISSN: 0044-605X. Publisher: Acta Veterinaria Scandinavica.

AB The purpose of this study was to evaluate blood chemical and clin. response of castrated young boars to com. available vaccines to E. coli where the bacteria have been formaldehyde killed and the endotoxins have not been removed. The animals that received the vaccine strictly s.c. did not show any clin. or blood biochem. changes as compared to a pig, which received the same dose i.v. Under clin. field circumstances the vaccinations are performed s.c./i.m. and the uptake from the injection site can vary. However, there is a risk of the vaccine coming directly into the circulation through small blood vessels. Since the boars received the same dose of the vaccines as recommended for pregnant gilts or sows in late pregnancy, the findings were discussed in terms of the risks that might be seen in pregnancy.

L35 ANSWER 6 OF 19 MEDLINE on STN

1998420426. PubMed ID: 9749978. Balance of proinflammatory and antiinflammatory cytokines in mice immunized with Escherichia coli and correlation with mortality after lethal challenge. Raponi G; Ghezzi M C; Lun M T; Mancini C. (I Chair of Clinical Microbiology, Faculty of Medicine, La Sapienza University of Rome, Italy.) Medical microbiology and immunology, (1998 Jun) Vol. 187, No. 1, pp. 11-6. Journal code: 0314524. ISSN: 0300-8584. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The balance of proinflammatory and antiinflammatory cytokines, their correlation with endotoxin levels and mortality rate after lethal challenge of Escherichia coli was investigated in mice immunized weekly for 8 weeks with formalin-killed E. coli

either untreated or treated with 0.5x minimal inhibitory concentration of aztreonam. Control mice treated in parallel with saline, died within 24 h after challenge with 100x lethal dose (LD50) of viable E. coli O6:K-. Mice immunized with antibiotic-treated bacteria showed a significantly higher survival than mice immunized with untreated E. coli. Cytokines were not detected in the sera of control mice during the entire period of immunization. At 90 min after immunization, mice immunized with antibiotic-treated E. coli showed tumor necrosis factor-alpha (TNF-alpha) levels significantly lower and interleukin (IL)-6 levels significantly higher ($P < 0.05$) than mice immunized with untreated E. coli, while comparable levels of interferon-gamma (IFN-gamma were measured in both groups. TNF-alpha and IL-10 levels measured 90 min after lethal challenge correlated with the mortality rate observed in each group ($r = 0.96$ for TNF-alpha and 0.94 for IL-10). IL-6 levels correlated with survival ($r = 0.95$), while IFN-gamma serum levels did not differ in the two immunized groups, but were significantly higher than those measured in the control mice. IL-4 was detected only after challenge of mice immunized with antibiotic-treated bacteria. Comparable levels of circulating endotoxin were measured after lethal challenge in both control and immunized mice. These data showed that in the presence of a protective immune response the survival of immunized mice was correlated with an early alteration of cytokine expression pattern including enhanced secretion of IL-4, IL-6 and IFN-gamma, and reduced secretion of TNF-alpha and IL-10.

L35 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 3

97316536. PubMed ID: 9172447. A 5-h screening and 24-h confirmation procedure for detecting Escherichia coli O157:H7 in beef using direct epifluorescent microscopy and immunomagnetic separation. Restaino L; Frampton E W; Irbe R M; Allison D R. (R & F Laboratories, West Chicago, IL 60185, USA.) Letters in applied microbiology, (1997 May) Vol. 24, No. 5, pp. 401-4. Journal code: 8510094. ISSN: 0266-8254. Pub. country: ENGLAND: United Kingdom. Language: English.

AB An antibody-direct epifluorescent filter technique (Ab-DEFT) detected 100% of the raw ground beef samples inoculated with Escherichia coli O157:H7 cells (0.15 cells g-1) and incubated in a prewarmed, modified buffered peptone water (mBPW) non-selective enrichment broth for 5 h at 42 degrees C in an orbital shaking water bath (200 rev min-1). Over 50% of the microscopic fields viewed were positive (1-10 fluorescent cells field-1) in the Ab-DEFT. All positive screening results were confirmed within 24 h by subjecting 1 ml of the mBPW to the Dynabeads anti-E. coli O157 immunomagnetic separation procedure, followed by plating on MacConkey sorbitol agar containing 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide. At this cell concentration, 41.7% of the inoculated samples were detected by the conventional method involving a 24-h selective enrichment. Exposure to viable cells before filtration was minimized by using a 0.58% formaldehyde concentration for 5 min at 50 degrees C (killed > 4.00 logs of E. coli O157:H7 cells) without affecting cell fluorescence.

L35 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN

1996:307731 Document No. 124:340906 Method for immunization of poultry with vaccines. Takeda, Reiji; Ekino, Shigeo; Sugimori, Giichi; Nakamura, Takashi; Aoyama, Shigemi (Shionogi Seiyaku Kk, Japan). Jpn. Kokai Tokkyo Koho JP 08073377 A2 19960319 Heisei, 6 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1995-169717 19950705. PRIORITY: JP 1994-153342 19940705.

AB A method for immunization of poultry with vaccines against e.g. Escherichia coli type O2 or Brucella abortus for infection prevention involves: administration of the vaccine (killed E. coli type O2 or B. abortus) to the excretory tract of fetuses during hatching. E.g. E. coli type O2 vaccine is prepared by cultivation of E. coli type O2 in BHI medium at 37° for 24 h, treatment of cultured E. coli type O2 with 0.2% formaldehyde at room temperature for 48 h,

suspension of the treated E. coli type O2 in 0.2%
saline to final concentration of 1.5 X 1010 CFU/mL, and finally sonication.

L35 ANSWER 9 OF 19 MEDLINE on STN DUPLICATE 4
95105015. PubMed ID: 7806373. Role of endotoxin in acute inflammation induced by gram-negative bacteria: specific inhibition of lipopolysaccharide-mediated responses with an amino-terminal fragment of bactericidal/permeability-increasing protein. Kohn F R; Kung A H. (XOMA Corporation, Berkeley, California 94710.) Infection and immunity, (1995 Jan) Vol. 63, No. 1, pp. 333-9. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB A recombinant 23-kDa amino-terminal fragment of human bactericidal/permeability-increasing protein (rBPI23), a potent lipopolysaccharide (LPS)-binding/neutralizing protein, was used as a probe to assess the role of endotoxin in the acute inflammatory responses elicited by gram-negative bacteria in rat subcutaneous air pouches. In initial experiments, rBPI23 prevented the Escherichia coli O111:B4 LPS-induced accumulation of polymorphonuclear leukocytes (PMN), tumor necrosis factor alpha (TNF-alpha), and nitrite (a stable end product of nitric oxide formation) in exudate fluids. Significant inhibition of TNF-alpha production was still evident when rBPI23 treatment was delayed for 30 min after LPS instillation. In subsequent experiments, rBPI23 also prevented the nitrite and early (2-h) TNF-alpha accumulation induced by three different strains of formaldehyde-killed gram-negative bacteria (E. coli O7:K1, E. coli O111:B4, and Pseudomonas aeruginosa 12.4.4) but did not inhibit the PMN or late (6-h) TNF-alpha accumulation induced by these bacteria. As with LPS challenge, a significant inhibition of early TNF-alpha production was still evident when rBPI23 treatment was delayed for 30 to 60 min after instillation of killed bacteria. The results indicate that in this experimental model the NO and early TNF-alpha responses to gram-negative bacterial challenge are mediated predominantly by endotoxin, whereas the PMN and late TNF-alpha responses may be mediated by other bacterial components. Moreover, the results indicate that rBPI23 can inhibit the bacterially induced production of certain potentially harmful mediators (TNF-alpha and NO) without entirely blocking the host defense, i.e., PMN response, against the bacteria.

L35 ANSWER 10 OF 19 MEDLINE on STN
96072457. PubMed ID: 8568283. In vivo chemoactivation of oyster hemocytes induced by bacterial secretion products. Alvarez M R; Friedl F E; Roman F R. (Department of Biology, University of South Florida, Tampa 33620-5150, USA.) Journal of invertebrate pathology, (1995 Nov) Vol. 66, No. 3, pp. 287-92. Journal code: 0014067. ISSN: 0022-2011. Pub. country: United States. Language: English.

AB Movements of tissue hemocytes in the Eastern oyster Crassostrea virginica were monitored and quantified by image analysis of sections following inoculation with agar cores containing Escherichia coli or cell-free medium on which the bacteria had previously grown. Hemocytes respond to the presence of live bacteria by accumulating in widely dispersed areas of tissue surrounding the gut and digestive diverticula. The response is rapid and evident within 40 min, is maximal at 1 hr, and declines by 3 hr after inoculation. Sterile implanted agar cores do not produce a response. Bacteria killed with ozone elicit a response when inoculated together with the medium on which they had grown while bacteria killed by heat or formalin do not. Killed bacteria suspended in saline fail to stimulate hemocyte chemokinesis. Cell-free medium applied externally produces a response equal to that measured with live bacteria inoculated internally. Extraction of bacteria-free medium with hexane does not significantly reduce hemocyte chemokinesis. Digestion of bacteria-free medium with pronase completely eliminates chemokinesis. Molecular filtrates of bacteria-free medium induce maximal chemokinetic response at molecular weight as low as 1 kDa. These data show that the oyster hemocyte activators produced by E. coli are most likely low-molecular-weight polypeptides which

diffuse from the site of inoculation and can pass through the intact external surface epithelium to induce a chemokinetic response.

L35 ANSWER 11 OF 19 MEDLINE on STN
95282476. PubMed ID: 7762266. Vaccination with a formalin-killed P-fimbriated E. coli whole-cell vaccine prevents renal scarring from pyelonephritis in the non-human primate. Roberts J A; Kaack M B; Baskin G; Svenson S B. (Department of Urology, Tulane University School of Medicine, New Orleans, LA 70112, USA.) Vaccine, (1995 Jan) Vol. 13, No. 1, pp. 11-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB A formalin-killed P-fimbriated Escherichia coli serotype O4 vaccine was evaluated for protective efficacy in monkeys in an experimental pyelonephritis model following urethral bacterial inoculation. The vaccination did not protect against initial colonization and there were no significant differences in the time of bacteriuria after experimental infection in the two groups of animals. The whole-cell vaccine offers a limited protection against renal dysfunction and scarring ($p = 0.002$) and less renal involvement ($p = 0.04$), results that are quite similar to those given by a synthetic O-antigen-specific saccharide-protein conjugate vaccine previously tested.

L35 ANSWER 12 OF 19 EMBASE
92219624 EMBASE Document No.: 1992219624. Oral vaccination of weaned rabbits against enteropathogenic Escherichia coli-like E. coli O103 infection: Use of heterologous strains harboring lipopolysaccharide or adhesin of pathogenic strains. Milon A.; Esslinger J.; Camguilhem R.. Departement de Biologie Moleculaire, Un. Associee Microbiol. Moleculaire, INRA, 23, Chemin des Capelles,F-31076 Toulouse Cedex, France. Infection and Immunity Vol. 60, No. 7, pp. 2702-2709 1992.
ISSN: 0019-9567. CODEN: INFIBR
Pub. Country: United States. Language: English. Summary Language: English.
Entered STN: 920816. Last Updated on STN: 920816
AB To test the importance of lipopolysaccharide (LPS) and adhesin as major antigens in vaccination against rabbit enteropathogenic Escherichia coli (EPEC)-like E. coli O103 infection, we used two nonpathogenic wild-type strains to immunize rabbits at weaning. One of these strains (C127) harbors the O103 LPS but does not express the 32,000-molecular-weight adhesin that characterizes the highly pathogenic O103 strains. The other (C6) belongs to the O128 serogroup, which does not cross-react with the O103 serogroup, but expresses the adhesin. These strains were administered orally, either live or after Formalin inactivation. After vaccination, the animals were challenged with highly pathogenic O103 strain B10. Compared with rabbits vaccinated with the Formalin-killed homologous strain, rabbits vaccinated with killed C127 or C6 showed partial but significant protection. When given live, these strains colonized more or less heavily the digestive tract of the animals and provided nearly complete (C127) or complete (C6) protection against challenge. They induced a quick local immune response, as judged by fecal immunoglobulin A anti-LPS kinetics. Furthermore, strain C6 induced an ecological effect of 'resistance to colonization' against challenge strain B10. This effect may have been due to the adhesin that is shared by both strains and to the production of a colicin. Strain C6 could inhibit in vitro the growth of highly pathogenic O103 strains. On the whole, our results show that adhesins and LPS are important, although probably not exclusive, protection-inducing components in rabbit EPEC-like colibacillosis and provide insight into possible protection of rabbits against EPEC-like E. coli infection with live strains.

L35 ANSWER 13 OF 19 MEDLINE on STN DUPLICATE 5
92189657. PubMed ID: 1799394. [Immunomodulating effect of killed , apathogenic Escherichia coli, strain Nissle 1917, on the macrophage system]. Immunmodulierende Wirkung von abgetoteten apathogenen

Escherichia coli, Stamm Nissle 1917, auf das Makrophagensystem. Hockertz S. (Fraunhofer Institut fur Toxikologie, Abteilung Immunbiologie/Immunotoxikologie, Hannover.) Arzneimittel-Forschung, (1991 Oct) Vol. 41, No. 10, pp. 1108-12. Journal code: 0372660. ISSN: 0004-4172. Pub. country: GERMANY: Germany, Federal Republic of. Language: German.

AB The influence of formaldehyde-killed Escherichia coli strain Nissle 1917 (SK 22) on macrophages of C57BL/6 mice was investigated in vitro. It has been shown that SK 22 activated macrophages derived from bone marrow produced Interleukin-6 with high efficiency. In addition, SK 22 stimulated macrophages to secrete tumor necrosis factor, as measured by a bioassay. Furthermore, macrophages were activated by SK 22 to produce a 3 fold amount of oxygen radicals compared to the spontaneous oxygen radical production. In contrast to this finding, the phagocytic capacity of these macrophages was only slightly increased. The specific lysis of P 815 tumor cells by peritoneal macrophages after coincubation with SK 22 was measured using tumor cells prelabelled with radioactive 51Cr. The results of the in vitro experiments presented clearly show that the E. coli preparation SK 22 is an efficient immunomodulator of the unspecific immune system.

L35 ANSWER 14 OF 19 MEDLINE on STN

85055122. PubMed ID: 6501409. Legionella pneumophila inhibits acidification of its phagosome in human monocytes. Horwitz M A; Maxfield F R. The Journal of cell biology, (1984 Dec) Vol. 99, No. 6, pp. 1936-43. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB We used quantitative fluorescence microscopy to measure the pH of phagosomes in human monocytes that contain virulent Legionella pneumophila, a bacterial pathogen that multiplies intracellularly in these phagocytes. The mean pH of phagosomes that contain live L. pneumophila was 6.1 in 14 experiments. In the same experiments, the mean pH of phagosomes containing dead L. pneumophila averaged 0.8 pH units lower than the mean pH of phagosomes containing live L. pneumophila, a difference that was highly significant (P less than 0.01 in all 14 experiments). In contrast, the mean pH of phagosomes initially containing live E. coli, which were then killed by monocytes, was the same as for phagosomes initially containing dead E. coli. The mean pH of L. pneumophila phagosomes in activated monocytes, which inhibit L. pneumophila intracellular multiplication, was the same as in nonactivated monocytes. To simultaneously measure the pH of different phagosomes within the same monocyte, we digitized and analyzed fluorescence images of monocytes that contained both live L. pneumophila and sheep erythrocytes. Within the same monocyte, live L. pneumophila phagosomes had a pH of approximately 6.1 and sheep erythrocyte phagosomes had a pH of approximately 5.0 or below. This study demonstrates that L. pneumophila is capable of modifying the pH of its phagocytic vacuole. This capability may be critical to the intracellular survival and multiplication of this and other intracellular pathogens.

L35 ANSWER 15 OF 19 MEDLINE on STN DUPLICATE 6

84238363. PubMed ID: 6376357. In vitro cytotoxic effect of alpha-hemolytic Escherichia coli on human blood granulocytes. Gadeberg O V; Orskov I. Infection and immunity, (1984 Jul) Vol. 45, No. 1, pp. 255-60. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The cytotoxic effect of Escherichia coli bacteria on human blood granulocytes was measured by recording numbers of nonlysed cells and percentages of viable cells after in vitro incubation with bacteria in the presence of plasma. A total of 179 strains from various sources of infection were tested. Of 117 alpha-hemolytic strains, 59 were cytotoxic. Five nonhemolytic mutant strains, derived from alpha-hemolytic cytotoxic strains, were nontoxic. None of the 62 nonhemolytic strains were toxic. Four spontaneously occurring alpha-hemolytic, nontoxic mutant strains were isolated from cytotoxic ones. Cytotoxicity of bacteria reached a maximum

after log-phase growth at 30 to 37 degrees C for 2.5 h, and the toxic capacity was equal after growth in various media, including human urine and plasma. The cytotoxic effect increased with the length of exposure of granulocytes to bacteria and with increasing numbers of bacteria per granulocyte. Cytotoxic strains showed different degrees of toxicity, highly cytotoxic strains lysing about 90% of the granulocytes and killing about one-half of nonlysed cells in 1 h. Bacteria killed by heat, formaldehyde, or UV light were nontoxic. Alpha-hemolytic strains of O groups 2, 4, 6, 25, and 75 originating from various infections in humans were more frequently cytotoxic than alpha-hemolytic strains of other O groups derived from human infections. Culture supernatants containing free alpha-hemolysin were highly cytotoxic to human blood granulocytes, monocytes, and lymphocytes in vitro, whether supernatants originated from cytotoxic or noncytotoxic bacteria. Cytotoxicity to phagocytes, which is mediated by or closely linked genetically to alpha-hemolysin, may be a mechanism by which alpha-hemolytic strains of E. coli strengthen their ability to establish and maintain infections.

L35 ANSWER 16 OF 19 MEDLINE on STN DUPLICATE 7

83056932. PubMed ID: 6815187. The role of phospholipase A2 lysines in phospholipolysis of Escherichia coli killed by a membrane-active neutrophil protein. Forst S; Weiss J; Elsbach P. The Journal of biological chemistry, (1982 Dec 10) Vol. 257, No. 23, pp. 14055-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Purified rabbit bactericidal/permeability-increasing protein at bactericidal concentrations is a membrane-perturbing agent that triggers hydrolysis of envelope phospholipids of a phospholipase A-less Escherichia coli (S17) mutant by a highly basic (pI greater than 10) phospholipase A2, purified from Agkistrodon halys blomhoffii snake venom. Most other purified phospholipases A2 do not degrade the phospholipids of E. coli killed by the bactericidal protein. To study the role of enzyme charge in bactericidal protein-dependent phospholipid hydrolysis, lysines of the Agkistrodon phospholipase A2 were modified, either by carbamylation (decreases net charge), or by reductive methylation (no delta charge). Incorporation of [14C]cyanate or [14C] formaldehyde and amino acid analysis served to monitor modification. Modification appears to be limited to epsilon-NH2 groups. Incorporation of up to 5 mol of cyanate or formaldehyde/mol of enzyme did not affect catalytic activity. In contrast, incorporation of, on average, 1 mol of either reagent/mol of protein reduced by 80% the activity of the enzyme toward E. coli S17 killed by the bactericidal protein. Since this loss is similar with carbamylation and reductive methylation, the role of the epsilon-NH2 group in the bactericidal protein-dependent hydrolysis seems independent of charge. Thus, the lysines in this phospholipase A2 are not essential for catalysis and substrate binding, but are essential for the action of this enzyme on E. coli killed by the bactericidal protein.

L35 ANSWER 17 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

1982:147297 Document No.: PREV198273007281; BA73:7281. LEAKAGE INDUCED IN ESCHERICHIA-COLI CELLS BY A PROTEIN RNA COMPLEXES FROM BACTERIO PHAGE F-2. DE MARS CODY J [Reprint author]; CONWAY T W. DEP BIOCHEM, UNIV IOWA, IOWA CITY, IOWA 52242, USA. Journal of Virology, (1981) Vol. 39, No. 1, pp. 60-66.

CODEN: JOVIAM. ISSN: 0022-538X. Language: ENGLISH.

AB Complexes of f2 phage RNA and its A protein, or maturation protein, transfect E. coli cells much better than protein-free RNA. These complexes were used to introduce the bactgeriophage f2 lysis gene into cells. The A protein-RNA complex killed cells, probably by causing them to leak large macromolecules. Previously induced β-galactosidase leaked from cells treated either with the A protein-RNA complex or with lethal but noninfectious complexes that had

been treated with formaldehyde. This observation was consistent with an earlier finding that formaldehyde-treated f2 RNA stimulates the in vitro synthesis of a lysis protein. The complexes did not stimulate the rate of leakage of β-galactosidase from a streptomycin-resistant mutant know to be lysis defective. The rate of leakage was increased in a double mutant resistant to both streptomycin and rifampin and which is lysed normally by f2 bacteriophage.

L35 ANSWER 18 OF 19 MEDLINE on STN
81152810. PubMed ID: 7010560. Protective effect of immunization with Salmonella minnesota Re 595 against ascending Escherichia coli O6K13H1 pyelonephritis in rats. Larsson P; Kaijser B; Baltzer I M; Olling S. Scandinavian journal of infectious diseases. Supplementum, (1980) Vol. Suppl 24, pp. 220-3. Journal code: 0251025. ISSN: 0300-8878. Pub. country: Sweden. Language: English.
AB Active as well as passive immunization with formalin-killed S. minnesota Re 595 bacteria protected against experimental, ascending pyelonephritis caused by E. coli O6K13H1 in rats. Absorption of the hyperimmune sera with Re antigen before passively given did not eliminate the protective effect. The specificity of protective antibodies is discussed.

L35 ANSWER 19 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
1978:127944 Document No.: PREV197865014944; BA65:14944. EFFECT OF HEAT ON ANTIGENICITY AND IMMUNOGENICITY OF THE ANTIGENIC DETERMINANT SHARED BY HAEMOPHILUS-INFLUENZAE TYPE B AND ESCHERICHIA-COLI K-100. WHANG H Y [Reprint author]; GOLDHAR J; NETER E. LAB BACTERIOL, CHILD HOSP, BUFFALO, NY 14222, USA. Infection and Immunity, (1977) Vol. 18, No. 1, pp. 68-72. CODEN: INFIBR. ISSN: 0019-9567. Language: ENGLISH.
AB E. coli K100 produces an antigenic determinant similar to or identical with the capsular antigen of H. influenzae type b. The effects of heat on the immunogenicity, erythrocyte-modifying capacity and antigenicity of this cross-reacting antigen (CRA) were studied. Immunization of rabbits with viable or formaldehyde-killed suspensions of E. coli K100, producing CRA, engendered CRA antibodies in significant titers, as demonstrated by hemagglutination of erythrocytes modified by H. influenzae type b antigen. Heating of the suspensions for 1 h at 56 or 100° C destroyed the immunogenicity of CRA, and the heated suspensions did not prime for a secondary antibody response. Supernatants of heated suspensions also were non-immunogenic. Repeated freezing and thawing of heated suspensions of E. coli K100 or their supernatants did not restore immunogenicity. Heat also abolished the immunogenicity of H. influenzae type b. Loss of immunogenicity of CRA of E. coli K100 by heat was not due to alteration of the antigenic determinant, since heated suspensions and supernatants thereof modified erythrocytes for agglutination by H. influenzae type b antiserum. The latter supernatants also inhibited hemagglutination by H. influenzae type b antibodies and absorbed the latter. Striking differences exist in the effects of heat on CRA compared to enterobacterial common antigen and lipopolysaccharide O antigen of enteric bacteria. Heating of the latter 2 antigens does not abolish their priming effect, and repeated freezing and thawing restores their immunogenicity.

=> s l11 and bleach
L36 1 L11 AND BLEACH

=> d l36 cbib abs

L36 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
2002:222954 Document No.: PREV200200222954. Lactic acid bacteria protect leaves from infection by environmental microbes. Gomez, L. [Reprint author]; Cheng, A.; Watkins, C.; Dahlen, C.; Vermeulen, C. W.. Division

Ave High School, Levittown, NY, USA. Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 508. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology. Orlando, FL, USA. May 20-24, 2001. American Society of Microbiology.
ISSN: 1060-2011. Language: English.

AB That tender, moist mesophyll cells remain uninfected although exposed to external microbes via a leaf's stomata prompted us to think that plants may be using the same protective strategy that mammalian portal membranes use - a coating of bacteria of that division of "lactics" called the dairy bacteria. Leaves and dairy bacteria have long been associated (silage), but what these few species of slow-growing fastidious bacteria are doing in plants has not been asked. Whole leaf squashes of surface-sterilized (bleach) leaves were made onto paper-covered replication blocks, and then printed onto various agars resulting in abundant growth of mixed populations. Numerous streptococci and lactobacilli were identified microscopically from colonies of different morphologies. Choosing a "universal" plant - the dandelion - we found that the bacterial subpopulations were species specific over a 150 mile range. Testing whether these bacteria merely filled a niche, or they actively protected the leaf was done both in situ and in vitro. Blue dye mixes of E .coli and B.subtilis were injected horizontally into the mesophyll of several leaves, and at timed intervals, the leaves were cracked open at the blue injection lines, and swabbings were plated on MacConky agar, or briefly boiled and plated on nutrient agar. Within 60 minutes, both bacteria were killed. An in vitro mixed culture of "leaf bacteria" in nutrient broth was also inoculated with low numbers of E.coli and B.subtilis. (Filtrates were not lethal to the "contaminants.") Again within an hour both became undetectible. Thus, not only are "lactics" copiously present INSIDE leaves, but they also have the capacity, presumably via bacteriocins, to kill other microbes common in the environment. Implications: (1) evolution - two kingdoms using the same protection strategy; and (2) agriculture - development of lactics with more potent bacteriocins.

=> s l11 and ozone
L37 32 L11 AND OZONE

=> dup remove l37
PROCESSING COMPLETED FOR L37
L38 15 DUP REMOVE L37 (17 DUPLICATES REMOVED)

=> d l38 1-15 cbib abs

L38 ANSWER 1 OF 15 MEDLINE on STN DUPLICATE 1
2006248007. PubMed ID: 16672466. Inactivation of enterohemorrhagic Escherichia coli in rumen content- or feces-contaminated drinking water for cattle. Zhao Tong; Zhao Ping; West Joe W; Bernard John K; Cross Heath G; Doyle Michael P. (Center for Food Safety, University of Georgia, Griffin, GA 30223, USA.) Applied and environmental microbiology, (2006 May) Vol. 72, No. 5, pp. 3268-73. Journal code: 7605801. ISSN: 0099-2240. Pub. country: United States. Language: English.

AB Cattle drinking water is a source of on-farm Escherichia coli O157:H7 transmission. The antimicrobial activities of disinfectants to control E. coli O157:H7 in on-farm drinking water are frequently neutralized by the presence of rumen content and manure that generally contaminate the drinking water. Different chemical treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate, and competing E. coli, were tested individually or in combination for inactivation of E. coli O157:H7 in the presence of rumen content. Chlorine (5 ppm), ozone (22 to 24 ppm at 5 degrees C), and competing E. coli treatment of water had minimal effects (<1 log CFU/ml reduction) on killing

E. coli O157:H7 in the presence of rumen content at water-to-rumen content ratios of 50:1 (vol/wt) and lower. Four chemical-treatment combinations, including (i) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (treatment A); (ii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (treatment B); (iii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (treatment C); and (iv) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 100 ppm chlorine dioxide (treatment D); were highly effective (>3 log CFU/ml reduction) at 21 degrees C in killing E . coli O157:H7, O26:H11, and O111:NM in water heavily contaminated with rumen content (10:1 water/rumen content ratio [vol/wt]) or feces (20:1 water/feces ratio [vol/wt]). Among them, treatments A, B, and C killed >5 log CFU E. coli O157:H7, O26:H11, and O111:NM/ml within 30 min in water containing rumen content or feces, whereas treatment D inactivated approximately 3 to 4 log CFU/ml under the same conditions. Cattle given water containing treatment A or C or untreated water (control) ad libitum for two 7-day periods drank 15.2, 13.8, and 30.3 liters/day, respectively, and cattle given water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 liters/day. The amounts of water consumed for all water treatments were significantly different from that for the control, but there were no significant differences among the water treatments. Such treatments may best be applied periodically to drinking water troughs and then flushed, rather than being added continuously, to avoid reduced water consumption by cattle.

L38 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

2006:1302956 Experimental observation on bactericidal efficacy of OM-500 ozone air disinfector. Luo, Jun; Long, Bei-guo; Long, Min; Lai, Jian-ping; Zhang, Wen-bing (Department of Microbiology, School of Public Health and Tropic Medicine, Southern Medical University, Guangzhou, 510515, Peop. Rep. China). Xiandai Yufang Yixue, 33(10), 1954-1955 (Chinese) 2006. CODEN: XYYIFS. ISSN: 1003-8507. Publisher: Xiandai Yufang Yixue Zazhishe.

AB Objective: To study the air disinfection effect of Om-500 ozone air disinfector using two methods. Methods: When carrier quant. test was used, we found that 94.41% of Staphylococcus aureus and 100% of E . coli was killed if Om-500 ozone air disinfector for 60 min, and 97.3% of E. coli was killed if operated for 5 min. When simulate locale air disinfection test was used, the result shows that decrease rate of nature bacterium in air can reach to 90.88% and 95.74% resp. Conclusion: The experiment suggested that it has a good air disinfection effect.

L38 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

2006:462613 Document No. 145:425652 Development of a supersonic levitation washer-disinfector using ozone micro-bubbling and silver electrolysis. Ueda, Toyotoshi; Hara, Masanori; Nishiyama, Kyohei; Ando, Satoru; Shimizu, Mitsuhiro; Shigihara, Takanori; Koshiba, Mamiko; Nakamura, Shun (Department of Chemistry, Faculty of Science and Engineering, Meisei University, 2-1-1, Hodokubo, Hino-shi, Tokyo, 191-8506, Japan). Bokin Bobai, 34(4), 201-209 (Japanese) 2006. CODEN: BOBODP. ISSN: 0385-5201. Publisher: Nippon Bokin Bobai Gakkai.

AB A new type of supersonic washer-disinfector using ozone micro-bubbling and silver electrolysis was developed in order to clean and disinfect many devices and materials such as semiconductors, endoscopes and cut vegetables. This washer has fourteen supersonic oscillators of an umbrella shape, which emit supersonic traveling waves along more than two directions and are driven independently by each supersonic transducer. This supersonic levitation washer can evenly clean not only hard materials such as glasses, jewels and metals, but also soft materials such as clothes, plastics, rubbers and bodies. Neither detergent nor disinfectant is necessary: therefore, its drainage does not cause environmental pollution. Disinfection is easy and rapid using ozone oxidation and silver electrolysis. Ozone is produced by the irradiation of UV

light to the atmospheric oxygen and jets as micro-bubbles. Electrolysis is carried out using a d.c. between the pos. electrode of a net-shaped silver plate and the neg. electrode of a stainless-steel container. E. coli (103-106 cells/mL) was killed within 20 min either by ozone or silver electrolysis. Bacillus atrophaeus (104 cells/mL) was killed in 30 min by ozone and in 5 min by silver electrolysis. S. cerevisiae (104 cells/mL) was killed in 1 min by silver electrolysis. This apparatus meets the new demand for cleaning with the conservation of the global environment in mind.

L38 ANSWER 4 OF 15 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 2

2004:983208 The Genuine Article (R) Number: 865BN. Electrochemical wastewater disinfection: Identification of its principal germicidal actions. Li X Y (Reprint); Diao H F; Fan F X J; Gu J D; Ding F; Tong A S F. Univ Hong Kong, Dept Civil Engn, Pokfulam Rd, Hong Kong, Hong Kong, Peoples R China (Reprint); Univ Hong Kong, Dept Civil Engn, Hong Kong, Hong Kong, Peoples R China; Tsing Hua Univ, Dept Environm Sci & Engn, Beijing, Peoples R China; Macao Water Supply Co Ltd, SAAM, Macau, Peoples R China; Univ Hong Kong, Dept Ecol & Biodivers, Hong Kong, Hong Kong, Peoples R China; Hong Kong SAR Govt, Environm Protect Dept, Hong Kong, Hong Kong, Peoples R China. xlia@hkucc.hku.hk. JOURNAL OF ENVIRONMENTAL ENGINEERING-ASCE (OCT 2004) Vol. 130, No. 10, pp. 1217-1221. ISSN: 0733-9372. Publisher: ASCE-AMER SOC CIVIL ENGINEERS, 1801 ALEXANDER BELL DR, RESTON, VA 20191-4400 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Laboratory experiments were carried out to investigate the mechanisms of electrochemical (EC) wastewater disinfection. Artificial wastewater contaminated by Escherichia coli (E. coli) culture, and which contained different salts of NaCl, Na2SO4, and NaNO3, was used as the test medium. The experimental results do not favor the hypotheses that the EC bactericidal action was due to cell destruction by the electric field and the production of persulfate. In comparison to direct chlorination, the EC process displayed a much stronger disinfecting capability than that of electrochlorination assumed for EC disinfection. Observations with scanning electron microscopy on the E. coli bacteria of wastewater treated by different means of disinfection suggested that the cells were likely killed during the EC treatment by chemical products with oxidizing and germicidal powers similar to that of ozone and much stronger than that of chlorine. All of the findings support the theory that the major killing function of EC disinfection is provided by short-lived and high-energy intermediate EC products, such as free radicals.

L38 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

2004:598356 Document No. 142:193766 Comparison of examination of germicidal efficacy of ozone water by two test methods. Jiang, Li; Wang, Taixing; Rao, Lin (Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China). Zhongguo Xiaoduxue Zazhi, 20(1), 11-13 (Chinese) 2003. CODEN: ZXZAFO. ISSN: 1001-7658. Publisher: Zhongguo Xiaoduxue Zazhi Bianjibu.

AB Suspension quant. germicidal test method and quant. germicidal test method using carriers immersed in running liquid disinfectant for examining efficacy of ozone water in killing Escherichia coli were compared and the influence of peptone on its germicidal efficacy was examined The results indicated that when suspension quant. germicidal test method was used, in absence of peptone, the ozone water containing ozone 8.0 mg/L with a 1 min contact time killed 100% of E. coli in average and if peptone 10 g/L was present, the same ozone water with a 10 min contact time killed 65.96% of E. coli in average When quant. germicidal test method using carriers immersed in running liquid disinfectant was used, the ozone water containing ozone 8.0 mg/L with a 10 min contact time killed 99.97% of E. coli in average and if the bacterial suspension contained higher than 25% volume of calf serum, the

germicidal efficacy was influenced significantly. The results suggest that when running ozone water is used in surface disinfection, examination of germicidal efficacy by quant. germicidal test method using carriers immersed in running liquid disinfectant relatively approximates the real condition.

L38 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
2002:964231 Document No. 138:44757 Medical devices treatment with ozone for prevention of infection. Darouiche, Rabih O.; Shannon, David C. (Baylor College of Medicine, USA). PCT Int. Appl. WO 2002100455 A2 20021219, 30 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US17806 20020605. PRIORITY: US 2001-296837P 20010608.

AB Indwelling medical devices resistant to microbial colonization and other complications include devices having a coating on 1 or more surfaces comprising an effective amount or concentration of an oxygen-releasing substance, such as ozone, and optionally, other therapeutic agents. Devices may alternately include a sleeve or other means which allows one or more surfaces of the device to be flushed or insufflated periodically with ozone or another oxygen-releasing substance. A clin. isolate of Escherichia coli strain 2131 that had caused catheter-related infection was used. In the exptl. arm, ozone was bubbled into the bacterial suspension. In the control arm, no ozone was bubbled. Ozone killed E. coli in solution

L38 ANSWER 7 OF 15 MEDLINE on STN DUPLICATE 3
2001335889. PubMed ID: 11403125. Inactivation of Escherichia coli O1 57:H7, Listeria monocytogenes, and Lactobacillus leichmannii by combinations of ozone and pulsed electric field. Unal R; Kim J G; Yousef A E. (Department of Food Science and Technology, The Ohio State University, Columbus 43210, USA.) Journal of food protection, (2001 Jun) Vol. 64, No. 6, pp. 777-82. Journal code: 7703944. ISSN: 0362-028X. Pub. country: United States. Language: English.

AB Pulsed electric field (PEF) and ozone technologies are nonthermal processing methods with potential applications in the food industry. This research was performed to explore the potential synergy between ozone and PEF treatments against selected foodborne bacteria. Cells of Lactobacillus leichmannii ATCC 4797, Escherichia coli O157:H7 ATCC 35150, and Listeria monocytogenes Scott A were suspended in 0.1% NaCl and treated with ozone, PEF, and ozone plus PEE Cells were treated with 0.25 to 1.00 microg of ozone per ml of cell suspension, PEF at 10 to 30 kV/cm, and selected combinations of ozone and PEF. Synergy between ozone and PEF varied with the treatment level and the bacterium treated. L. leichmannii treated with PEF (20 kV/cm) after exposure to 0.75 and 1.00 microg/ml of ozone was inactivated by 7.1 and 7.2 log10 CFU/ml, respectively; however, ozone at 0.75 and 1.00 microg/ml and PEF at 20 kV/cm inactivated 2.2, 3.6, and 1.3 log10 CFU/ml, respectively. Similarly, ozone at 0.5 and 0.75 microg/ml inactivated 0.5 and 1.8 log10 CFU/ml of E. coli, PEF at 15 kV/cm inactivated 1.8 log10 CFU/ml, and ozone at 0.5 and 0.75 microg/ml followed by PEF (15 kV/cm) inactivated 2.9 and 3.6 log10 CFU/ml, respectively. Populations of L. monocytogenes decreased 0.1, 0.5, 3.0, 3.9, and 0.8 log10 CFU/ml when treated with 0.25, 0.5, 0.75, and 1.0 microg/ml of ozone and PEF (15 kV/cm), respectively; however, when the bacterium was treated with 15 kV/cm, after exposure to 0.25, 0.5, and 0.75 microg/ml of ozone, 1.7, 2.0, and 3.9 log10 CFU/ml were killed, respectively. In conclusion, exposure of L. leichmannii, E. coli, and L. monocytogenes to ozone followed by the PEF treatment showed a synergistic bactericidal effect. This synergy was most apparent with mild doses of ozone against L. leichmannii.

L38 ANSWER 8 OF 15 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 4

2001224959 EMBASE Impairment of microbial killing and superoxide-producing activities of alveolar macrophages by a low level of ozone.
Mochitate K.; Katagiri K.; Miura T.. K. Mochitate, Environ. Health Sciences Division, Natl. Inst. for Environ. Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-0053, Japan. mochitat@nies.go.jp. Journal of Health Science Vol. 47, No. 3, pp. 302-309 2001.
Refs: 30.
ISSN: 1344-9702. CODEN: JHSCFD
Pub. Country: Japan. Language: English. Summary Language: English.
Entered STN: 20010717. Last Updated on STN: 20010717

AB Male Wistar rats were exposed to 0.2 ppm ozone for up to 14 days, during which alveolar macrophages were collected by pulmonary lavage to assess the effect of ozone on their microbial killing and superoxide-producing activities. For rapid assessment of microbial killing activity, we measured the release of (3)H-radioactivity into the supernatant by deoxycholate-lysis of the macrophages that had phagosytosed and killed (3)H-uridine-labeled microbes. The killing activity against Escherichia coli and Candida albicans was reduced to 70-80% of control levels on day 3. However, phagocytosis by and the activity of lysosomal enzymes of the macrophages were not impaired. On day 14 the killing activity against E. coli had returned to control levels, whereas that against C. albicans was still reduced. Because active oxygen species plays an important role in microbial killing activity of macrophages, the effects of ozone on respiratory burst and superoxide production were examined. Aliquots of alveolar macrophages were stimulated with phorbol myristate acetate (PMA), opsonized zymosan, or lipopolysaccharide (LPS) plus cytochalasin E (Cyt.E). The respiratory burst, oxygen consumption for rapid superoxide production, was decreased to 60-80% of control levels on day 3. On day 14, the respiratory burst by opsonized zymosan was still 80% reduced, whereas that by PMA or LPS plus Cyt.E had returned to control levels. In addition, the superoxide-producing activity of ozone-exposed macrophages was 10-60% decreased on day 3. On day 14, the superoxide production by stimulation with opsonized zymosan was still 60% reduced, whereas that by PMA or LPS plus Cyt.E had returned to control levels. In conclusion, because of their decreased production of superoxide, the host defense activity of alveolar macrophages was impaired by in vivo exposure to 0.2 ppm ozone. In particular, the C. albicans-associated defect lasted throughout the exposure period.

L38 ANSWER 9 OF 15 MEDLINE on STN

2001396497. PubMed ID: 11447890. Reviewing efficacy of alternative water treatment techniques. Hambidge A. Health estate, (2001 Jun) Vol. 55, No. 6, pp. 23-5. Journal code: 100888268. Pub. country: England: United Kingdom. Language: English.

AB This section is designed to provide a brief summary of some of the findings. A good deal of work has been conducted by Mr N. L. Pavey and the team at BSRIA, Bracknell. The BSRIA publications are an excellent source of further information. Ultraviolet radiation: UV radiation of wavelength 254 nm destroys bacteria by a mechanism of damaging nucleic acids by producing thymine dimers which disrupt DNA replication [Gavdy and Gavdy, 1980]. L. pneumophila has been reported as sensitive to UV dosages of 2,500-7,000 uW.s/cm2 [Antopol & Ellner, 1979; Knudson, 1985]. Antopol and Ellner [1979] examined the susceptibility of L. pneumophila to UV dosage. Their results indicated that 50% of the organisms were killed by 380 uWs/cm2 and 90% were killed by 920 uWs/cm2. Kills of 99 and 99.9% were obtained using 1,840 and 2,760 uWs/cm2 respectively. Muraca et al [1987] showed that continuous UV irradiation resulted in a 5 logarithm decrease in waterborne L. pneumophila in a circulating system. Gilpin [1984] reported that in laboratory buffer solutions, exposure to 1 uW of UV radiation per cm2 achieved a 50% kill of L longbeachae in 5 minutes, L. gormanii in 2-30

minutes and L pneumophila in 17 minutes. Exposure times for 99% kills for L. longbeachae, L pneumophila and L. Gormanii were 33, 48 and 63 minutes respectively. The same research worker conducted experiments using a 3 litre circulating water system, connected to a stainless steel housing containing a UV source. The UV lamp output was 7 ergs/mm2 per second per 100 cm at 254 nm. L. pneumophila was killed within 15 seconds, that is within their first pass through the system. Continuous disinfection with UV has the advantages of imparting no taste, odour or harmful chemical by-products and requires minimal operation and maintenance [Muraca et al 1988]. Keevil et al [1989] state that UV irradiation fails to clear systems of biofilm because of poor penetration into microflocs of the micro-organisms. Copper/silver ionisation: A recent study of full scale hot water test rigs incorporating copper-silver ionisation systems has been reported by Pavey, 1996. Copper and silver ions were introduced into the water by electrolysis. One of the principal mechanisms of biocidal action of these ions is thought to be cell penetration. The positively charged copper ions form electrostatic bonds with negatively charged sites on the cell wall. The cell membrane is thus distorted, allowing ingress of silver ions which attack the cell by binding at specific sites to DNA, RNA, respiratory enzymes and cellular protein, causing catastrophic failure of the life support systems of the cell. Silver and copper ion concentrations of 40 and 400 ug/L respectively were effective against planktonic Legionellae in cold water systems and hot water systems containing soft water. In hard water, the ionisation was ineffective due to the inability to control silver ion concentrations. This was caused by scaling of the electrodes and silver ion complexation by the high concentration of dissolved solids. Bosch et al [1993] had earlier extended the application of copper-silver disinfection to human enteric viruses in water, such as adenovirus, rotavirus, hepatitis A virus, and poliovirus. Their work showed that copper and silver ions in the presence of reduced levels of free chlorine did not ensure the total elimination of viral pathogens from water. In the case of an amoeba, Naegleria fowleria [responsible for primary amoebic meningoencephalitis], Cassells et al [1995] have demonstrated that a combination of silver and copper ions were ineffective at inactivating the amoebae at 80 and 800 ug/L respectively. However addition of 1.0 mg/L free chlorine produced a synergistic effect, with superior inactivation relative to either chlorine or silver-copper in isolation. A similar synergy was reported by Yahya et al [1989] in their study of Staphylococcus sp. and Pseudomonas aeruginosa. Yahya et al [1992] also suggested an additive or synergistic effect in the inactivation of coliphage MS-2 and poliovirus. Other techniques: There are a number of other techniques. We have conducted trials of most of these in the control of Legionella sp., but these fall out of the scope of this article, and as such less emphasis has been placed on them here. Ozonation: Ozone [O3] is an oxidising gas, generated electrically from oxygen [O2]. L. pneumophila can be killed at < 1 mg/L of ozone [Edelstien et al 1982]. Muraca et al [1987] found that 1-2 mg/L of continuous ozone over a six hour contact time, produced a 5 logarithm decrease of L. pneumophila. The effectiveness of ozone treatment against a range of bacteria and coliphages has been studied Botzenhart et al [1993]. E. coli was least resistant to ozone, followed by MS 2-coliphage and PhiX 174-coliphage, with L. pneumophila and Bacillus subtilis spores being the most resistant. (ABSTRACT TRUNCATED)

L38 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

1999:347130 Document No. 131:184089 Disinfection of fresh vegetables by various means and the bactericidal effects of strong acidic electrolyzed solution on enteropathogenic bacteria. Ueda, Shigeko; Kuwabara, Yoshihiro (Hygiene Laboratory, Kagawa Nutrition University, Saitama, Sakado-shi, Chiyoda, 350-0214, Japan). Bokin Bobai, 27(5), 301-307 (Japanese) 1999. CODEN: BOBODP. ISSN: 0385-5201. Publisher: Nippon Bokin Bobai Gakkai.

AB Five kinds of fresh salad vegetables such as parsley, sprouts, sani-lettuce, cabbage and cucumber were washed and disinfected for 5 min

in various disinfectants and detergents including strong or weak acidic electrolyzing solns., ozone-water, hypochlorite solution, acetic acid solution, com. detergents and so on. Total aerobic bacterial counts of vegetables were depressed more effectively by these treatments than by washing only with tap water. Particularly, the treatment with a strong acidic electrolyzing solution decreased the bacterial counts by about 10-2cfu/g on all vegetables. Furthermore, among 17 kinds of vegetables treated with strong acidic electrolyzed solution, cabbage, sani-lettuce, lettuce, spinach and parsley had decreases in bacterial counts in the ranges of 10-2-103cuff/g. Similarly, coliforms, faecal E. coli and B. cereus were shown to decrease in number on all of vegetables after the treatment. The bacterial effects of strong acidic electrolyzing solns. and hypochlorite solns. with the same levels of active chlorine on various types of bacterial were examined Although gram neg. bacterial were killed within 3 min and staphylococci were completely killed within 30 s after exposure to the acidic electrolyzing solution, the time needed to kill spore-forming bacteria was more than 5 min. The bactericidal activity of strong acidic electrolyzing solns. was shown to be relatively higher than that of hypochlorite solns.

L38 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN
1999:502120 Document No. 132:89295 Experimental observation on germicidal efficacy of TT-100-type ozone disinfector. Liao, Ruyan; Lin, Jinyan; Chen, Wensheng; Huang, Xianzhong; Chen, Hongmin (Guangdong Provincial Sanitary and Anti-Epidemic Station, Canton, 510300, Peop. Rep. China). Zhongguo Xiaoduxue Zazhi, 16(2), 84-87 (Chinese) 1999. CODEN: ZXZAFO. ISSN: 1001-7658. Publisher: Zhongguo Xiaoduxue Zazhi Bianjibu.
AB TT-100-type Ozone Disinfector generates ozone 1.50-1.62 mg/min in average The killing rates of Escherichia coli and Staphylococcus aureus in artificially contaminated water were 100% after introduction of ozone generated by operation of the disinfector for 5 min and 10 min into the water resp. The killing rate of Bacillus subtilis var. niger spores in water was only 81.23% after introduction of ozone for 30 min. Immersion of bacteria carriers made by different materials and contaminated with E. coli or S. aureus in water which was then treated with introduction of ozone for 15 min killed more than 99.9% of the bacteria on surfaces of aluminum and glass carriers and less than 99.9% of the bacteria on cloth and paper carriers. Natural bacteria test indicated that immersion of spinach and apples in water which was then treated with introduction of ozone for 15 min killed more than 94.9% of the natural bacteria on their surfaces. Introduction of ozone generated by disinfector for 15 min into a 18 m3 unoccupied room could reduce the total air bacteria count by 83.83%.

L38 ANSWER 12 OF 15 MEDLINE on STN DUPLICATE 5
96035678. PubMed ID: 7574656. Efficacy of ozonated water against various food-related microorganisms. Restaino L; Frampton E W; Hemphill J B; Palnikar P. (R & F Laboratories, Inc., Bridgeview, Illinois 60455, USA.) Applied and environmental microbiology, (1995 Sep) Vol. 61, No. 9, pp. 3471-5. Journal code: 7605801. ISSN: 0099-2240. Pub. country: United States. Language: English.
AB The antimicrobial effects of ozonated water in a recirculating concurrent reactor were evaluated against four gram-positive and four gram-negative bacteria, two yeasts, and spores of Aspergillus niger. More than 5 log units each of Salmonella typhimurium and Escherichia coli cells were killed instantaneously in ozonated water with or without addition of 20 ppm of soluble starch (SS). In ozonated water, death rates among the gram-negative bacteria--S. typhimurium, E. coli, Pseudomonas aeruginosa, and Yersinia enterocolitica--were not significantly different ($P > 0.05$). Among gram-positive bacteria, Listeria monocytogenes was significantly $P < 0.05$) more sensitive than either Staphylococcus aureus or Enterococcus faecalis. In the presence of organic material, death rates of S. aureus compared with L. monocytogenes and E. coli compared with S. typhimurium in ozonated

water were not significantly (P > 0.05) affected by SS addition but were significantly reduced (P < 0.05) by addition of 20 ppm of bovine serum albumin (BSA). More than 4.5 log units each of Candida albicans and Zygosaccharomyces bailii cells were killed instantaneously in ozonated water, whereas less than 1 log unit of Aspergillus niger spores was killed after a 5-min exposure. The average ozone output levels in the deionized water (0.188 mg/ml) or water with SS (0.198 mg/ml) did not differ significantly (P < 0.05) but were significantly lower in water containing BSA (0.149 mg/ml).

L38 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 6

96072457. PubMed ID: 8568283. In vivo chemoactivation of oyster hemocytes induced by bacterial secretion products. Alvarez M R; Friedl F E; Roman F R. (Department of Biology, University of South Florida, Tampa 33620-5150, USA.) Journal of invertebrate pathology, (1995 Nov) Vol. 66, No. 3, pp. 287-92. Journal code: 0014067. ISSN: 0022-2011. Pub. country: United States. Language: English.

AB Movements of tissue hemocytes in the Eastern oyster Crassostrea virginica were monitored and quantified by image analysis of sections following inoculation with agar cores containing Escherichia coli or cell-free medium on which the bacteria had previously grown. Hemocytes respond to the presence of live bacteria by accumulating in widely dispersed areas of tissue surrounding the gut and digestive diverticula. The response is rapid and evident within 40 min, is maximal at 1 hr, and declines by 3 hr after inoculation. Sterile implanted agar cores do not produce a response. Bacteria killed with ozone elicit a response when inoculated together with the medium on which they had grown while bacteria killed by heat or formalin do not. Killed bacteria suspended in saline fail to stimulate hemocyte chemokinesis. Cell-free medium applied externally produces a response equal to that measured with live bacteria inoculated internally. Extraction of bacteria-free medium with hexane does not significantly reduce hemocyte chemokinesis. Digestion of bacteria-free medium with pronase completely eliminates chemokinesis. Molecular filtrates of bacteria-free medium induce maximal chemokinetic response at molecular weight as low as 1 kDa. These data show that the oyster hemocyte activators produced by E. coli are most likely low-molecular-weight polypeptides which diffuse from the site of inoculation and can pass through the intact external surface epithelium to induce a chemokinetic response.

L38 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

1992:423156 Document No. 117:23156 Growth delay and inactivation of intracellular catalase of resting Escherichia coli K-12 cells exposed to ozone. Shimada, Keiko; Takahashi, Minako; Shimahara, Kenzo (Eng. Coll., Seikei Univ., Tokyo, Japan). Seikei Daigaku Kogakubu Kogaku Hokoku, 53, 3607-8 (Japanese) 1992. CODEN: SKKGAW. ISSN: 0582-4184.

AB An E. coli culture in a 100 μM ozone solution lost .apprx.90% of its catalase activity and >99% of the cells were killed. Repeated exposure (5 min each) of the cells to fresh solns. of ozone resulted in more extensive killing than a single exposure.

L38 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2007 ACS on STN

1963:54974 Document No. 58:54974 Original Reference No. 58:9438a-c Effect of ozone on survival and permeability of Escherichia coli. McNair Scott, D. B.; Lesher, E. C. (Univ. of Pennsylvania, Philadelphia). Journal of Bacteriology, 85, 567-76 (Unavailable) 1963. CODEN: JOBAAY. ISSN: 0021-9193.

AB E. coli cultures in the logarithmic phase or resting were treated with various concns. of ozone in saline solution Approx. 2 + 107 mols. of ozone per bacterium killed 50% of the cells. Ozone caused leakage of cell content into the medium, and lysis of some cells. Low concns. did not react with the glutathione within the cells, although reaction with

glutathione in solution was immediate and stoichiometric. The effect on nucleic acid within the cells was to change the solubility and to cause the release of ultraviolet-absorbing material into the medium. Ozone attacked the ring structure of the base or the carbohydrate only when the substance was in the medium. Nucleic acids released into the medium were reabsorbed by cells which were not, lysed. Viable cells resumed growth immediately, and grew at rates determined by the nutrients either added to the medium or which resulted from leakage and lysis of nonviable cells. It is postulated that the primary attack of ozone was on the cell wall or membrane of the bacteria, probably by reaction with the double bonds of lipids, and that leakage or lysis of the cells depended on the extent of that reaction.

=> s l11 adn alcohol
MISSING OPERATOR L11 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l11 and alcohol
L39 29 L11 AND ALCOHOL

=> dup remove l39
PROCESSING COMPLETED FOR L39
L40 19 DUP REMOVE L39 (10 DUPLICATES REMOVED)

=> d l40 1-19 cbib abs

L40 ANSWER 1 OF 19 MEDLINE on STN DUPLICATE 1
2003003037. PubMed ID: 12475283. Green-leaf-derived C6-aroma compounds with potent antibacterial action that act on both Gram-negative and Gram-positive bacteria. Nakamura Soichiro; Hatanaka Akikazu. (Department of Life and Environmental Sciences, Shimane University, Shimane 690-8550,. Japan.soichiro@edu.shimane-u.ac.jp) . Journal of agricultural and food chemistry, (2002 Dec 18) Vol. 50, No. 26, pp. 7639-44. Journal code: 0374755. ISSN: 0021-8561. Pub. country: United States. Language: English.
AB All eight C6-aliphatic alcohol and aldehyde compounds in naturally occurring green leaves showed bacteriostatic effects against Staphylococcus aureus IFO 12732, methicillin-resistant S. aureus, Escherichia coli IFO 3301, E. coli O157:H7, and Salmonella enteritidis, with bacteriostatic activities of less than 12.5 microg mL(-1). In this study, the susceptibility of Gram-positive bacteria tested was observed to be greater than that of Gram-negative bacteria. The bactericidal action of the aldehyde compounds was found to be much stronger than that of the alcohol compounds under both liquid and gaseous conditions. The most effective compound was (3E)-hexenal at concentrations of 0.1 and 1 microg mL(-1), which killed 2.1 x 10(5) cfu mL(-1) of S. aureus IFO 12732 and 1.4 x 10(5) cfu mL(-1) of E. coli IFO 3301, respectively, by direct contact with the compound. Lethality of (3E)-hexenal against S. aureus IFO 12732 and E. coli IFO 3301 was also observed as a result of gaseous contact at concentrations of 3 and 30 microg mL(-1), respectively. The bactericidal effects of 30 microg mL(-1) (3E)-hexenal were thoroughly maintained throughout periods of 2 days and 1 day against S. aureus IFO 12732 and E. coli IFO 3301, respectively, by a complex formation with alpha-cyclodextrin.

L40 ANSWER 2 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
2002:369022 The Genuine Article (R) Number: 543LZ. Liver sinusoidal endothelial cell injury by neutrophils in rats with acute obstructive cholangitis. Gong J P (Reprint); Wu C X; Liu C A; Li S W; Shi Y J; Li X H; Peng Y. Chongqing Univ Med Sci, Coll Clin Med 2, Dept Gen Surg, 74 Linjuang Rd, Chongqing 400010, Peoples R China (Reprint); Chongqing Univ Med Sci, Coll Clin Med 2, Dept Gen Surg, Chongqing 400010, Peoples R

China; Chongqing Univ Med Sci, Affiliated Hosp 2, Chongqing 400010, Peoples R China. WORLD JOURNAL OF GASTROENTEROLOGY (APR 2002) Vol. 8, No. 2, pp. 342-345. ISSN: 1007-9327. Publisher: W J G PRESS, PO BOX 2345, BEIJING 100023, PEOPLES R CHINA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB AMI: The objective of this study is to elucidate the potential role of poly-morphonuclear neutrophils (PMN) in the development of such a sinusoidal endothelial cell (SEC) injury during early acute obstructive cholangitis (AOC) in rats.

METHODS: Twenty one Wistar rats were divided into three groups: the ACC group. the bile duct ligated group (BDL group), and the sham operation group (SO group), The common bile duct (CBD) of rats in ACC group was dually ligated and 0. 2 ml of the E. coli O-111 B-4 (5 x 10(9) cfu/ml) suspension was injected into the upper segment, in BDL group, only the CBD was ligated and in SO group. neither injection of E. coli suspension nor CBD ligation was done. but the same operative procedure. Such group consisted of seven rats, all animals were killed 6 h after the operation. Morphological changes of the liver were observed under light and electron microscope. Expression of intercellular adhesion molecule-1 (ICAM-1) mRNA in hepatic tissue was determined with reverse transcription polymerase chain reaction (RT-PCR). The serum levels of alanine aminotransferase (ALT) were determined with anutoanalyger and cytokine-induced neutrophil chemoattractant (CINC) was determined by enzyme-linked immunosorbent assay (ELISA).

RESULTS: Neutrophils was accumulated in the hepatic sinusoids and sinusoidal endothelial cell injury existed in ACC group. In contrast, in rats of BDL group, all the features of SEC damage were greatly reduced, Expression of ICAM-1 mRNA in hepatic tissue in three groups were 7.54 +/- 0, 82, 2. 87 +/- 0. 34, and 1. 01 +/- 0. 12, respectively. There were significant differences among three groups P < 0. 05). The serum CINC levels in the three groups were 188 +/- 21 ng . L-1, 94 +/- 11 ng . L-1, and 57 +/- 8 ng . L-1, respectively. There were also significant differences among the three groups (P < 0. 05). Activity of the serum ALT was 917 &PLUSMN; 167 nkat &BULL; L-1. 901 &PLUSMN; 171 nkat &BULL; L-1. and 908 &PLUSMN; 164 nkat &BULL; L-1. respectively, (P > 0.005).

CONCLUSION: Hepatic SEC injury occurs earlier than hepatic parenchymal cells during AOC. Recruitments of circulating neutrophils in the hepatic sinusoidal space might mediate the SEC injury, and ICAM-1 in the liver may modulate the PMN of accumulation.

L40 ANSWER 3 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2001:421969 The Genuine Article (R) Number: 431MG. Wine has activity against entero-pathogenic bacteria in vitro but not in vivo. Sugita-Konishi Y (Reprint); Hara-Kudo Y; Iwamoto T; Kondo K. Natl Inst Infect Dis, Dept Biomed Food Res, Shinjuku Ku, 1-23-1 Toyama, Tokyo 1628640, Japan (Reprint); Natl Inst Infect Dis, Dept Biomed Food Res, Shinjuku Ku, Tokyo 1628640, Japan; Natl Inst Hlth & Nutr, Dept Food Sci, Shinjuku Ku, Tokyo 1628640, Japan; Ochanomizu Univ, Fac Human Life & Environm Sci, Bunkyou Ku, Tokyo 1128610, Japan. BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY (APR 2001) Vol. 65, No. 4, pp. 954-957. ISSN: 0916-8451. Publisher: JAPAN SOC BIOSCI BIOTECHN AGROCHEM, JAPAN ACAD SOC CTR BLDG, 2-4-6 YAYOI BUNKYO-KU, TOKYO, 113, JAPAN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We studied the activity of wine against entero-pathogenic bacteria both lit vitro and in vivo, The food-borne bacteria were killed in both red and white wine within 30 min. However the results of a Salmonella infection experiment using mice suggested that wine was not effective e in preventing food-borne diseases in vivo.

L40 ANSWER 4 OF 19 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

2001:768601 The Genuine Article (R) Number: 474KC. Role of the outer membrane of Eschericia coli AG100 and Pseudomonas aeruginosa NCTC 6749 and

resistance/susceptibility to monoterpenes of similar chemical structure. Griffin S G (Reprint); Wyllie S G; Markham J L. Univ Western Sydney, Ctr Biostruct & Biomol Res, Richmond, NSW 2753, Australia (Reprint). JOURNAL OF ESSENTIAL OIL RESEARCH (SEP-OCT 2001) Vol. 13, No. 5, pp. 380-386. ISSN: 1041-2905. Publisher: ALLURED PUBL CORP, 362 S SCHMALE RD, CAROL STREAM, IL 60188-2787 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Four pairs of oxygenated terpenes, with closely related chemical structures but considerably different minimum inhibitory concentration values (1) against F. aeruginosa or E. coh, showed differences in rate of cells killed over 2 h. Addition of polymyxin B nonapeptide (PMBN) as an outer membrane permeabilising agent was found to significantly increase the initial rates and overall numbers of cells killed for all compounds, The hydrocarbon limonene and the ester geranyl acetate, normally inactive, were also investigated. Both compounds had little killing effect when added alone to the cells but did show an enhanced killing capacity upon the addition of PBMN.

L40 ANSWER 5 OF 19 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
1999:466466 Document No.: PREV199900466466. Survival of bacteria at a subfreezing temperature (-1degreeC). Tanaka, Yoshinori [Reprint author]; Ishino, Tsuyoshi [Reprint author]; Matsuba, Takashi [Reprint author]; Takayama, Hisao [Reprint author]; Ishida, Shigeru. Department of Bacteriology, Faculty of Medicine, Tottori University, Yonago, 683-0826, Japan. Yonago Acta Medica, (July, 1999) Vol. 42, No. 2, pp. 147-152. print.
CODEN: YOAMAQ. ISSN: 0513-5710. Language: English.

AB Preservation of foodstuffs at temperatures around -1degreeC has attracted special interest recently. We investigated whether bacteria contaminating foodstuffs, especially contaminating fish, were killed or survived at -1degreeC compared with 37degreeC. Survival rates of Escherichia coli K12 and Staphylococcus aureus IFO12732 in nutrient broth at -1degreeC for 7 days were 52% and 31%, respectively. However, the survival rate of Vibrio parahaemolyticus in nutrient broth containing 3% NaCl at -1degreeC for 7 days was only 0.03%. When the bacteria were kept in a soy sauce solution containing alcohol and some seasonings (the soy sauce solution) at -1degreeC, survival rates of E. coli K12 and S. aureus IFO12732 after 2 days were 56% and 54%, respectively, but V. parahaemolyticus was completely killed after 24 h at -1degreeC in the soy sauce solution. When E. coli K12 and O157 and V. parahaemolyticus were incubated at -1degreeC in the soy sauce solution containing some pieces of raw fish (the improved soy sauce solution), 3 strains of the bacteria were not killed. These results indicate that bacteria contaminating fish are not killed at -1degreeC and that storage of fish at -1degreeC is not always effective in diminishing food poisoning.

L40 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1998:642268 Document No. 129:274986 Reduction of Escherichia coli O157:H7 population in soy sauce, a fermented seasoning. Masuda, Susumu; Hara-Kudo, Yukiko; Kumagai, Susumu (Noda Inst. Sci. Res., Noda, 278, Japan). Nippon Shoyu Kenkyusho Zasshi, 24(5), 275-281 (Japanese) 1998.
CODEN: NSKZDP. ISSN: 0286-7958. Publisher: Nippon Shoyu Kenkyusho.

AB The pathogenic E. coli, E. coli O157:H7, was added to soy sauce, and the effect of soy sauce against the growth of O157:H7 was examined The incubation at 30°C in soy sauce diminished the viable cells for 9 days. The bactericidal effect of soy sauce against the O157 strain was dependent on the temperature condition. The bactericidal effect was weak at 18°C, and there was no cell reduction effect at below 4°C. Even at the low temps., the O157 did not propagate in the soy sauce. Soy sauce was bactericidal at high temps. and bacteriostatic at low temps. Those effect of soy sauce against O157:H7 was dependent on the NaCl concentration, alc. concentration, pH, the kinds of organic acids, addition of preservatives, temps., and treatment time. Soy sauce

products confirmed to be safe from the contamination of E. coli O157:H7, because the bacterium will be killed by the soy sauce making processes such as fermentation, aging, and sterilization. As soy sauce products are preserved and sold at an ambient temperature, there is no chance for the bacterium to grow in the soy sauce products.

L40 ANSWER 7 OF 19 MEDLINE on STN DUPLICATE 2

91042421. PubMed ID: 2146486. Alcohol treatment of defective lambda lysogens is deletionogenic. Hayes S; Duncan D; Hayes C. (Department of Microbiology, College of Medicine, University of Saskatchewan, Saskatoon, Canada.) Molecular & general genetics : MGG, (1990 Jun) Vol. 222, No. 1, pp. 17-24. Journal code: 0125036. ISSN: 0026-8925. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB We ascertained that transient exposure to ethanol, above 18%, was deletionogenic to an Escherichia coli strain with a fragment (12.5 kb) of bacteriophage lambda integrated within the chromosome. The lambda attL B.P' through P fragment provided a forward selection for mutants, and a target for mutagenesis. The cells were killed by thermal derepression of transcription and replication of the lambda fragment when transferred from 30 degrees to 42 degrees C. Survivor mutants, capable of forming colonies at 42 degrees C, were selected from untreated starting cells. About half no longer supported marker rescue of the lambda fragment imm lambda (immunity) region, comprising the cI repressor, and the PL and PR promoters. Ethanol treatment of starting cells increased the occurrence of imm lambda-defective clones to near 100%. The mutations responsible for the imm lambda defect were found to be large deletions (12 kb or more of DNA). Ethanol treatment of the starting cells also produced a 5- to 18-fold increase in the occurrence of E. coli pgl mutations, which likely arose by the deletion mechanism generating the imm lambda defects, since pgl was closely linked to the integrated lambda fragment. A unifying hypothesis for these observations was that ethanol was deletionogenic. The inclusion or substitution of the int-kil segment of the lambda fragment produced no real change in the spontaneous occurrence of large imm lambda deletions from the untreated cells. Substitution of this segment suppressed the deletionogenic effect of ethanol, implying a prerequisite for sequence homology or gene function from this interval.(ABSTRACT TRUNCATED AT 250 WORDS)

L40 ANSWER 8 OF 19 MEDLINE on STN DUPLICATE 3

88258341. PubMed ID: 3290376. Efficacy of various methods of sterilization of acupuncture needles. Sisco V; Winters L L; Zange L L; Brennan P C. (Department of Microbiology, National College of Chiropractic, Lombard, IL 60148.) Journal of manipulative and physiological therapeutics, (1988 Apr) Vol. 11, No. 2, pp. 94-7. Journal code: 7807107. ISSN: 0161-4754. Pub. country: United States. Language: English.

AB The iatrogenic transmission of hepatitis B virus by inadequately sterilized acupuncture needles recently has been reported. Because some licensed chiropractors use acupuncture as a therapeutic modality, we have evaluated sterilization methods for these needles, which would be adaptable for use in a chiropractic office. Dry heat, boiling water, pressurized steam, sodium hypochlorite, and 70% alcohol were compared with a glass bead dry heat sterilizer originally developed for dental instruments. Presterilized acupuncture needles were contaminated with Bacillus stearothermophilus, Escherichia coli or Staphylococcus epidermidis and sterilized for intervals ranging from 5 sec to 30 min. The needles were then cultured to determine the efficacy of the sterilization regimen. Seventy percent alcohol was ineffective as a sterilization method. In terms of both time and convenience, the glass bead apparatus was the most efficient of the remaining methods tested. B. stearothermophilus-contaminated acupuncture needles were sterilized within 10 sec of exposure to preheated glass beads. Less than 10 sec exposure killed E. coli and S. epidermidis. A significant advantage of the glass bead sterilizer over the other methods was the absence of physical damage to the needles.

L40 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1987:418941 Document No. 107:18941 A molecular analysis of the RK mutatest. Gordon, Alasdair J. E.; Glickman, Barry W. (Dep. Biol., York Univ., Toronto, ON, M3J 1P3, Can.). Mutation Research, 190(4), 253-8 (English) 1987. CODEN: MUREAV. ISSN: 0027-5107.
AB The replicative killing (RK) test for detection of mutagens (in which Escherichia coli cells are killed by derepression of λ-DNA fragment based on temperature ≥39°) has been reported to show mutagenesis from EtOH; the RK tester strains CHY832 and SA431 of E. coli were hybridized with viral DNA. The expected extents of λ-DNA fragments in the strains were found but the complete mechanism of the RK mutatest is not understood.

L40 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1985:73951 Document No. 102:73951 Alcohol-induced suppression of the humoral immune response. Stolen, J. S.; Draxler, S.; Nagle, J. J. (Northeast Fish. Cent., Natl. Mar. Fish. Serv., Highlands, NJ, 07732, USA). Bulletin of Environmental Contamination and Toxicology, 34(1), 106-8 (English) 1985. CODEN: BECTA6. ISSN: 0007-4861.
AB Summer flounder (Paralichthys dentatus) pretreated with EtOH [64-17-5] or with EtOH + Aroclor 1254 [11097-69-1] showed a complete suppression of the immune response to formalin-killed human enteric Escherichia coli cells after their injection for 42 days. In nonpretreated fish agglutinating antibodies to E. coli were detected after 7 days. The EtOH + Aroclor 1254 pretreatment had a more pronounced immunosuppression that EtOH alone.

L40 ANSWER 11 OF 19 MEDLINE on STN DUPLICATE 4
84128718. PubMed ID: 6199044. Changes in streptonigrin lethality during adaptation of Escherichia coli to picolinic acid. Correlation with intracellular picolinate and iron uptake. Yeowell H N; White J R. Biochimica et biophysica acta, (1984 Mar 1) Vol. 797, No. 3, pp. 302-11. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
AB Uptake studies with [14C]picolinate and 55Fe3+ have provided an explanation for the change in streptonigrin killing on adaptation of Escherichia coli to picolinate, in terms of the available iron within the cell. When picolinic acid is added to a growing culture of E. coli an interval of bacteriostasis ensues; this adaptation period is followed by resumption of exponential growth. Addition of picolinate (4 mM) to a log phase culture of strain W3110 gave protection from the lethal action of streptonigrin (30 microM) when the two agents were added simultaneously. In contrast streptonigrin killed cells that had adapted to picolinate; however, a preincubation of adapted W3110 with phenethyl alcohol protected the cells from streptonigrin lethality. [14C]Picolinate uptake studies showed that initially picolinate entered the cells, but that it was excluded from adapted cells; addition of phenethyl alcohol permitted the entry of picolinate into adapted W3110. The changes in streptonigrin killing parallel the changes in concentration of intracellular picolinate, which can chelate the iron required by streptonigrin for its bactericidal action. 55Fe3+ uptake studies showed that initially picolinate prevented iron accumulation by strain W3110, whereas adapted cells did take up iron in the presence of picolinate. Addition of phenethyl alcohol prevented any observed uptake of iron by adapted W3110. This modulation of iron transport by picolinate also affects streptonigrin lethality. Experiments with iron transport mutants showed that picolinate acted on both the enterochelin and citrate routes of uptake. Therefore picolinate affects the concentration of available iron within the cell both by (a) its intracellular presence resulting in chelation of iron and (b) its action on iron uptake; these effects explain the change in streptonigrin killing on adaptation of E. coli to picolinate.

L40 ANSWER 12 OF 19 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights

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78282008 EMBASE Document No.: 1978282008. Possible sources of ethanol ante- and post-mortem: Its relationship to the biochemistry and microbiology of decomposition. Corry J.E.L.. Metrop. Police Forens. Sci. Lab., London, United Kingdom. Journal of Applied Bacteriology Vol. 44, No. 1, pp. 1-56 1978.
CODEN: JABAA4
Pub. Country: United Kingdom. Language: English.

AB Although ethanol can on rare occasions be detected in blood from living subjects who have not ingested alcohol, these levels never exceed 5 mg/100 ml. On the other hand, levels up to 150 mg/100 g have been detected in blood and tissues of putrefied human or rodent corpses. Ingestion of ethanol ante-mortem in these cases is known not to have taken place (in the case of the rodents), or is most unlikely to have taken place (in the case of humans). Production of ethanol has occurred, not only in tissues that have obviously putrefied, but within a relatively short time if temperatures are elevated (i.e. above about 15°C). Experience with decomposition of meat shows that high numbers of bacteria can be present without showing obvious signs of putrefaction. The limited evidence available suggests that ethanol is not formed post-mortem except by microbial action, and that ethanol is both produced and utilized, so that bodies with high initial levels will show a decrease, and bodies with low initial levels will show an increase. The method by which bacteria invade dead bodies is not entirely clear. However, the source appears to be mainly intestinal, although injury resulting in skin breakage immediately before death may introduce exogenous micro-organisms into the blood stream and throughout the body. There is evidence that bacteria may penetrate the intestinal walls during death and be distributed throughout the tissues in the blood stream, this may also occur during food absorption and from skin abrasions, etc. throughout life. Even after clinical death has occurred these organisms may be prevented from multiplying or actually killed, by the residual antimicrobial defences of the body, and the anaerobic organisms will be inhibited initially by the high Ph, but within a few hours, provided the temperature exceeds about 5°C, they will start to multiply. This primary invasion is probably reinforced by a secondary invasion of intestinal organisms, starting via the hepatic portal vein and the intestinal lymph system, and spreading round the body via the vascular system. Although the intestine harbours a wide variety of organisms, the majority obligate and fastidious anaerobes, only relatively few groups have been implicated as major colonizers of corpses during putrefaction; these include, in order of importance, Cl. perfringens (a vigorous saccharolytic, lipolytic and proteolytic organism) and other Clostridium spp., enterobacteria (frequently, E. coli and Proteus spp.), Micrococcaeae (frequently Staph. aureus), streptococci and Bacillus spp. All of these are capable of producing ethanol from glucose and other substrates. In addition, a wider variety of organisms may be detected in the early stages of putrefaction, and these include yeasts, which may produce very high ethanol levels if present in sufficiently high numbers. Information on levels of substrates present shortly after death is sparse and further studies on this subject would be of interest. Glucose may be present in high levels in the liver and nearby blood and tissues, levels in the blood generally may be raised. Other possibly important sources are amino-acids (especially once proteolysis has commenced), glycerol (formed during fat hydrolysis), and lactate which occurs widely and at levels over 100 mg/100 g in all tissues. There is evidence that all these compounds can serve as substrates for ethanol production by bacteria commonly found in corpses. Forensic scientists must, therefore, always bear in mind that specimens of human tissue containing micro-organisms, particularly specimens taken from corpses, may contain ethanol produced by microbial fermentation, and that extreme caution should be exercised when assessing the significance of post-mortem ethanol.

L40 ANSWER 13 OF 19 EMBASE

78161559 EMBASE Document No.: 1978161559. Enhanced susceptibility of mice to combinations of Δ9 tetrahydrocannabinol and live or killed gram negative bacteria. Bradley S.G.; Munson A.E.; Dewey W.L.; Harris L.S.. Dept. Microbiol., Med. Coll. Virginia, Virginia Commonwlth. Univ., Richmond, Va. 23298, United States. Infection and Immunity Vol. 17, No. 2, pp. 325-329 1977.
CODEN: INFIBR
Pub. Country: United States. Language: English.

AB Combinations of Δ9-tetrahydrocannabinol (Δ9-THC) and bacterial endotoxin were shown to be hyperadditively toxic for mice. A variety of purified lipopolysaccharide (LPS) preparations elicited enhanced mortality in combination with Δ9-THC. Escherichia coli O26:B6 LPS (Boivin preparation) at an essentially nonlethal dose of 2.5 mg/kg reduced the dose of Δ9-THC required to kill 50% of the treated mice from ca. 350 to 150 mg/kg. Inbred BALB, DBA, and C3H/HeCr mice, noninbred ICR mice, and hybrid CDF1 and BDF1 mice were hyperactive to combinations of Δ9-THC and LPS. Moreover, a variety of heat- killed intestinal and gram-negative bacteria, live E. coli, and complexes of lipid A with a variety of proteins substituted for LPS in the synergistic toxicity of LPS and Δ9-THC. Extracts of marijuana also elicited hyperreactivity to LPS. The hyperadditive lethality of combinations of Δ9-THC and LPS was markedly less in mice rendered refractory to LPS or Δ9-THC by repeated administration of LPS or Δ9-THC, respectively.

L40 ANSWER 14 OF 19 EMBASE

74186265 EMBASE Document No.: 1974186265. The photosensitizing action of carcinogens. I. The action of 2 naphthylamine on Escherichia coli K 12 and Paramecium caudatum. Ellis S.P.; Smith R.C.; Neely W.C.. Dept. Chem., Auburn Univ., Auburn, Ala. 36830, United States. Canadian Journal of Microbiology Vol. 20, No. 2, pp. 125-129 1974.
CODEN: CJMIAZ
Language: English.

AB Cultures of Paramecium caudatum incubated with 7 x 10-7 M 2 naphthylamine were rapidly killed when exposed to light of 366 nm. Cultures not exposed to the amine were unaffected by the light; cultures kept in the dark were unaffected by the amine. Escherichia coli K 12 populations were markedly reduced after irradiation of suspensions in water containing 3 x 10-4 M 2 naphthylamine with light simulating natural sunlight in intensity and wavelength distribution. Suspensions of E. coli in deionized water were unaffected by the light and E . coli suspended in solutions of the amine but kept in the dark were also unaffected. Since 2 naphthylamine is a known water pollutant, these results may be of ecological importance.

L40 ANSWER 15 OF 19 EMBASE

75004075 EMBASE Document No.: 1975004075. The immunologic role of the ethanol soluble enterobacterial common antigen versus experimental renal infection. McLaughlin J.C.; Domingue G.J.. Dept. Surg., Sect. Urol., Tulane Univ. Sch. !ed., New Orleans, La. 70112, United States. IMMUNOL.COMMUN. Vol. 3, No. 1, pp. 51-75 1974.
CODEN: XXXXXB
Language: English.

AB Members of the Enterobacteriaceae contain a common antigen (CA) which is found in the ethanol soluble fraction (ESF) of heat killed culture supernates. The ESF of an E. coli 06 strain was shown to be virtually endotoxin free. Preliminary chemical studies revealed that the dry ESF, including salts, contained 20% protein and less than 1% carbohydrate. Chloroform/methanol soluble lipid accounted for approximately 2% of the material. Vaccination of rabbits with such enterobacterial CA elicited protection against renal disease due to retrograde challenge with Proteus mirabilis or to hematogenous challenge with E. coli 075. Protection was not demonstrated

against a heavily encapsulated strain of Klebsiella pneumoniae which, as demonstrated by in vitro phagocytosis, was not opsonized by antibody to CA. These results suggest that further investigation of enterobacterial CA as a vaccine is warranted.

L40 ANSWER 16 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1967:516075 Document No. 67:116075 Disinfecting action of 4-(p-dialkylaminophenyl)pyridine derivatives. Kolomoitsev, L. R.; Alfer'eva, M. A.; Matvienko, N. I.; Sheinkman, A. K. (Donets'k. Med. Inst., Donetsk, USSR). Mikrobiologichnii Zhurnal (1934-1977), 29(4), 342-4 (Ukrainian) 1967. CODEN: MZUKAV. ISSN: 0026-3664.
AB The antibacterial properties of 4-(p-dibutylaminophenyl)pyridine-HCl (I), 4-(p-dimethylaminophenyl)pyridine-MeI (II), and N-(β-hydroxyethyl)-4-(p-dimethylaminophenyl)pyridinium chloride (III) were tested against Escherichia coli, Staphylococcus aureus, Bacillus anthracoides, and Bact. proteus vulgaris [Proteus vulgaris]. The prepns. are yellow crystalline powders, slightly soluble in water, but readily soluble in alc. The solns. of the prepns. do not decompose when heated to 100°, and do not lose their bactericidal properties. They are only slightly toxic. The prepns. were used in concns. of 2, 1, 0.5, and 0.25% in water. Test objects saturated with the bacterial suspensions were seeded on nutritive media containing the prepns. studied and observed for 24 hrs. I in 0.25% concentration sterilized the test objects infected with E. coli , S. aureus, and B. anthracoides within 15 min.; Proteus vulgaris were more resistant and were killed within about 6 hrs. All of the microorganisms were resistant to II and III, and were killed only after a longer period of time.

L40 ANSWER 17 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1957:48410 Document No. 51:48410 Original Reference No. 51:8989a-c Changes caused by injurious agents in the permeability of surviving cells of liver and kidney. Opie, Eugene L. (Rockefeller Inst. Med. Research, New York, NY). Journal of Experimental Medicine, 104, 897-919 (Unavailable) 1956. CODEN: JEMEAV. ISSN: 0022-1007.
AB cf. C.A. 50, 15808c. Slices of liver and kidney, immersed in oxygenated buffered Krebs-Ringer solution at 38°, were exposed to a variety of chemical and phys. agents and their permeability (I) measured. I was increased under conditions in which N replaced O, the temperature was raised to 58°, EtOH was added to make a dilution of 1/100-1/5000 (but not 1/20,000), the mol. concentration was raised 2-fold by NaCl, and when one of the
following was added: CHCl3, a filtrate of Escherichia, coli in a final dilution of 1/10,000, somatic antigen of Shigella paradysenteriae in a dilution of 1/1000-1/100,000, a suspension of killed typhoid bacilli of 1/1000-1/100,000 dilution, and urea in concns. of 0.01-0.1M. I was not increased by the addition of glucose, histamine, diphtheria toxin, or an E. coli filtrate of 1/10,000 dilution in the presence of a 2-fold increase in mol. concentration

L40 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1956:49234 Document No. 50:49234 Original Reference No. 50:9507d-h A comparative biochemical and immunological study of the directed mutability in some bacteria from the intestines. Belozerskii, A. N.; Spirin, A. S.; Kudlai, D. G.; Skavronskaya, A. G. (Moscow State Univ.). Biokhimiya (Moscow), 20, 686-95 (Unavailable) 1955. CODEN: BIOHAO. ISSN: 0320-9725.
AB Studies were conducted with (1) Escherichia coli, strain CM, grown in glucose-free Tyrode medium in the presence of heat-killed Salmonella paratyphi; (2) S. breslau Number 70; (3) Alkaligenes 11-IV-4 which was evolved from E. coli CM by culturing the latter in the presence of heat-killed S. Breslau Number 70; and (4) S. paratyphi mutant 12-IV-4 evolved from culturing Alkaligenes 11-IV-4 on synthetic medium in the presence of heat-killed S. breslau Number 70. All 4 types of bacteria were grown in parallel series on portions of the same batch of the same type of culture medium at 37° for 20 hrs. Growth was washed off with saline, washed again with saline,

alcohol and ether, and vacuum dried. The chemical characteristics of the corresponding bacterial masses were established by analyzing them for total N, total P, for purine base N, pentoses, reducing substances (after 4 hrs. hydrolysis with 1 N HCl), for total nucleic acids, deoxyribonucleic acid, ribonucleic acid, protein and polysaccharides. E. coli grown on synthetic medium in the presence of heat-killed S. breslau Number 70 undergoes basic mutation changes which are reflected in its chemical composition and immunological (antigenic) properties. Alkaligenes evolved from E. coli acquires a chemical and immunological entity all its own. The new strain which is evolved from the newly developed Alkaligenes strain, when again grown in the presence of heat-killed S. breslau Number 70 is a paratyphoid type of mutant, the chemical and immunologic characteristics of which are partly those of the original S. breslau and partly those of the Alkaligenes. The chemo-immunological analysis of the fractions indicated a phylogenetic connection between the experimentally evolved types and the original cultures. The nature of the chemical and immunological changes which had taken place present evidence of the complex structure of the protoplasm of the intestinal microorganisms under study. Among the protein complexes of the bacterial cells are found two specifically distinct components, one labile which changes from one biological form to another within narrow specific ramifications and a protoplasmic component more stable and equally specific within the ramifications of a broader systematic group.

L40 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2007 ACS on STN
1954:18847 Document No. 48:18847 Original Reference No. 48:3458d-f The use of metabolites in the restoration of the viability of heat and chemically inactivated Escherichia coli. Heinmets, F.; Taylor, W. W.; Lehman, J. J. (Naval Med. Research Lab., Camp Lejeune, NC). Journal of Bacteriology, 67, 5-12 (Unavailable) 1954. CODEN: JOBAAY. ISSN: 0021-9193.
AB Suspensions of E. coli, strain B/r, which had apparently been killed by the action of heat, Cl2, zephiran chloride, alc., or H2O2, were found to contain viable cells when incubated with various metabolites of the tricarboxylic series. When the apparently sterile suspensions were incubated in buffer or in nutrient broth, no viable cells could be demonstrated. The following metabolites were the most effective in producing reactivation: (1) with heat-"killed" cells, Na citrate, lactic acid, and oxalacetic acid; (2) Cl2-"killed" cells, Na citrate, malic acid, and oxalacetic acid; (3) H2O2-"killed" cells, Na citrate, lactic acid, and cis-aconitic acid; (4) zephiran chloride-"killed" cells, Na citrate, lactic acid, cis-aconitic acid, and isocitric acid; (5) alc.-"killed" cells, cis-aconitic acid, α-ketoglutaric acid, and succinic acid. The combination of 11 metabolites produced the highest reactivation. It is probable that such reactivation is concerned with resynthesis of enzymes and re-establishment of cyclic processes. Conventional testing and culturing are not adequate to determine complete sterility.

=> s allergen expressing bacteria
L41 0 ALLERGEN EXPRESSING BACTERIA

=> s allergen expressing E coli
L42 0 ALLERGEN EXPRESSING E COLI

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L43 1416 CAPLAN M?/AU

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L44 1 L43 AND MICROBIAL DELIVERY

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L44 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2007 ACS on STN
AN 2001:676622 CAPLUS
DN 135:225857
TI Microbial delivery system
IN Caplan, Michael
PA Panacea Pharmaceuticals, LLC, USA
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 14

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001066136	A2	20010913	WO 2000-US33121	20001206
	WO 2001066136	A3	20011227		
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	CA 2403292	A1	20010913	CA 2000-2403292	20001206
	AU 2001019510	A5	20010917	AU 2001-19510	20001206
	EP 1272213	A2	20030108	EP 2000-982485	20001206
	EP 1272213	B1	20060308		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
	JP 2004527450	T	20040909	JP 2001-564788	20001206
	PT 1272213	T	20060630	PT 2000-982485	20001206
	AU 765211	B2	20030911	AU 2001-43769	20010508
PRAI	US 2000-195035P	P	20000406		
	AU 1996-72433	A3	19960923		
	US 2000-731375	A	20001206		
	WO 2000-US33121	W	20001206		

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L46 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2007 ACS on STN
2005:259357 Document No. 142:334946 Recombinant allergens with mutated IgE epitopes for treating anaphylaxis induced by food, venom, drug and latex allergens. Caplan, Michael J.; Bottomly, Kim H.; Sosin, Howard B.; Burks, A. Wesley; Sampson, Hugh A. (USA). U.S. Pat. Appl. Publ. US 2005063994 A1 20050324, 117 pp., Cont.-in-part of U.S. Ser. No. 100,303. (English). CODEN: USXXCO. APPLICATION: US 2004-899551 20040726. PRIORITY: US 2000-195035P 20000406; US 2000-731375 20001206; US 2002-100303 20020318.
AB The present invention provides methods and compns. for treating or preventing allergic reactions, particularly anaphylactic reactions. Methods of the present invention involve administering microorganisms to allergic subjects, where the microorganisms contain a recombinant version of the protein allergen. The recombinant version can be wild-type or may include mutations within IgE epitopes of the protein allergen. Preferably the compns. are administered rectally. Particularly preferred microorganisms are bacteria such as E. coli . Any

allergen may be used in the inventive methods. Particularly preferred allergens are anaphylactic allergens including protein allergens found in foods, venoms, drugs and latex. The inventive compns. and methods are demonstrated in the treatment of peanut-induced anaphylaxis.

L46 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 1
1999394992. PubMed ID: 10464133. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. Caplan M S; Miller-Catchpole R; Kaup S; Russell T; Lickerman M; Amer M; Xiao Y; Thomson R Jr. (Department of Pediatrics, Northwestern University Medical School, Evanston Hospital, Evanston, Illinois, USA.) Gastroenterology, (1999 Sep) Vol. 117, No. 3, pp. 577-83. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.
AB BACKGROUND & AIMS: Neonatal necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease of premature infants partly caused by intestinal bacterial proliferation. Because bifidobacteria are thought to reduce the risk for intestinal disturbances associated with pathogenic bacterial colonization, we hypothesized that exogenous bifidobacterial supplementation to newborn rats would result in intestinal colonization and a reduction in the incidence of neonatal NEC. METHODS: Newborn rat pups were given Bifidobacterium infantis (10(9) organisms per animal daily), Escherichia coli, or saline control and exposed to the NEC protocol consisting of formula feeding (Esbilac; 200 cal. kg(-1). day(-1)) and asphyxia (100% N(2) for 50 seconds followed by cold exposure for 10 minutes). Outcome measures included stool and intestinal microbiological evaluation, gross and histological evidence of NEC, plasma endotoxin concentration, intestinal phospholipase A(2) expression, and estimation of intestinal mucosal permeability. RESULTS: Bifidobacterial supplementation resulted in intestinal colonization by 24 hours and appearance in stool samples by 48 hours. Bifidobacteria-supplemented animals had a significant reduction in the incidence of NEC compared with controls and E. coli-treated animals (NEC, 7/24 B. infantis vs. 19/27 control vs. 16/23 E. coli; $P < 0.01$). Plasma endotoxin and intestinal phospholipase A(2) expression were lower in bifidobacteria-treated pups than in controls, supporting the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC. CONCLUSIONS: Intestinal bifidobacterial colonization reduces the risk of NEC in newborn rats.

L46 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 2
95073279. PubMed ID: 7982271. Altered mitochondrial redox responses in gram negative septic shock in primates. Simonson S G; Welty-Wolf K; Huang Y T; Griebel J A; Caplan M S; Fracica P J; Piantadosi C A. (Department of Medicine, Duke University Medical Center, Durham, NC 27710.) Circulatory shock, (1994 May) Vol. 43, No. 1, pp. 34-43. Journal code: 0414112. ISSN: 0092-6213. Pub. country: United States. Language: English.
AB Gram negative sepsis causes changes in oxygen supply-demand relationships. We have used a primate model of hyperdynamic gram negative sepsis produced by intravenous infusion of Escherichia coli (E. coli) to evaluate sepsis-induced alterations in mitochondrial oxidation-reduction (redox) state in muscle in vivo. The redox state of cytochrome a,a3, the terminal member of the intramitochondrial respiratory chain, was assessed in the intact forearm by near-infrared (NIR) spectroscopy. The muscle NIR data were compared to routine measures of oxygen delivery (DO2) and oxygen consumption (VO2). After E. coli infusion and fluid resuscitation, DO2 and VO2 showed minimal changes through 24 hr of sepsis. In contrast, changes in cytochrome a,a3 redox state evaluated by NIR occurred within a few hours and were progressive. Mitochondrial functional responses were correlated with structural changes observed on serial muscle biopsies. Gross morphological changes in muscle mitochondria were present in some animals as early as 12 hr, and, in most animals, by 24 hr. The morphologic changes were consistent with decreases in oxidative capacity as suggested by NIR spectroscopy. The NIR data also suggest that two mechanisms are operating to explain abnormalities in oxygen metabolism and mitochondrial

function in lethal sepsis. These mechanisms include an early defect in oxygen provision to mitochondria that is followed by a progressive loss in functional cytochrome a,a3 in the muscle.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	387.36	387.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-24.96	-24.96

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Rapid publication

Persistent protective effect of heat-killed *Escherichia coli* producing "engineered," recombinant peanut proteins in a murine model of peanut allergy

Xiu-Min Li, MD,[a] Kamal Srivastava, MPhil,[a] Alexander Grishin, PhD,[a] Chih-Kang Huang, MS,[a] Brian Schofield, JD,[b] Wesley Burks, MD,[c] and Hugh A. Sampson, MD[a]

New York, NY, Baltimore, Md, and Little Rock, Ark

Background: Peanut allergy (PNA) is a life-threatening food allergy for which there is no definitive treatment.
Objective: We investigated the long-term immunomodulatory effect of heat-killed *Escherichia coli* producing engineered (mutated) Ara h1, 2, and 3 (HKE-MP123) administered rectally (pr) in a murine model of PNA.
Methods: Peanut-allergic C3H/HeJ mice received 0.9 (low dose), 9 (medium dose), or 90 (high dose) μg HKE-MP123 pr, HKE-containing vector (HKE-V) alone, or vehicle alone (sham) weekly for 3 weeks. Mice were challenged 2 weeks later. A second and third challenge were performed at 4-week intervals.
Results: After the first challenge, all 3 HKE-MP123 and HKE-V–treated groups exhibited reduced symptom scores ($P < .01$, .01, .05, .05, respectively) compared with the sham-treated group. Interestingly, only the medium- and high-dose HKE-MP123–treated mice remained protected for up to 10 weeks after treatment accompanied by a significant reduction of plasma histamine levels compared with sham-treated mice ($P < .05$ and .01, respectively). IgE levels were significantly lower in all HKE-MP123–treated groups ($P < .001$), being most reduced in the high-dose HKE-MP123–treated group at the time of each challenge. IL-4, IL-13, IL-5, and IL-10 production by splenocytes of high-dose HKE-MP123–treated mice were significantly decreased ($P < .01$; .001, .001, and .001, respectively), and IFN-γ and TGF-β production were significantly increased ($P < .001$ and .01, respectively) compared with sham-treated mice at the time of the last challenge.
Conclusions: Treatment with pr HKE-MP123 can induce long-term "downregulation" of peanut hypersensitivity, which might be secondary to decreased antigen-specific T_H2 and increased T_H1 and T regulatory cytokine production. (J Allergy Clin Immunol 2003;112:159-67.)

Key words: Peanut allergy, murine model, novel immunotherapy, persistent effect

Food allergy affects up to 6% of children younger than 4 years of age and about 2% of the US population beyond the first decade of life.[1] It is the single leading cause of anaphylaxis treated in hospital emergency departments in the United States and many "westernized" countries. Extrapolating from a survey in Olmstead County, Minnesota,[2] it is estimated that food allergy accounts for approximately 30,000 anaphylactic reactions and 150 deaths each year in the United States, with peanut (PN) and tree nut allergies accounting for the most severe reactions.[3,4] A national survey indicated that approximately 3 million Americans are allergic to PNs, tree nuts, or both.[5] Because of the apparent increasing prevalence of food allergy and the frequency and severity of food-induced anaphylactic reactions, food allergy research is receiving increased attention. However, there is still no treatment for this disorder.[6]

Antigen-specific immunotherapy is used to generate tolerance to specific allergens, but unlike traditional immunotherapy for inhalant and bee sting allergy, the benefit/risk ratio for injections of standard PN extracts was found to be unacceptable.[7] In a trial of standard rush immunotherapy in 6 patients with peanut allergy (PNA), Nelson and colleagues[7] observed an adverse reactions rate of 23% during the "buildup" phase and 39% during the "maintenance" phase, rates higher than those seen with aeroallergens and insect venom. Consequently, other forms of immunotherapy have been sought to treat food allergy.

From the [a]Department of Pediatrics, Mount Sinai School of Medicine, New York, [b]Johns Hopkins University Bloomberg School of Public Health, Baltimore, [c]Department of Pediatrics, Arkansas Children's Hospital Research Institute, University of Arkansas.

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Reprint requests: Hugh A. Sampson, MD, Department of Pediatrics; Box 1198, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029-6574.

0091-6749/2003 $30.00 + 0
doi:10.1067/mai.2003.1622

Abbreviations used
CT: Cholera toxin
DNP: Dinitrophenol
HKE: Heat-killed *Escherichia coli*
HKE-MP123: A mixture of equal proportions of HKE-mAra h1, HKE-mAra h2, and HKE-mAra h3
HKLM: Heat-killed *Listeria monocytogenes*
ig: Intragastric
MP123: A mixture of equal proportions of mAra h1, mAra h2, and mAra h3
PNA: Peanut allergy
PN: Peanut
pr: Per rectal
sc: Subcutaneously
SPC: Splenocyte

One approach for PNA is to generate engineered (mutated) PN proteins, modified (m)Ara h1, mAra h2, and mAra h3 (mAra-h123) in which IgE-binding epitopes have been altered by a critical amino acid to eliminate, or drastically reduce, IgE binding to the protein and therefore reduce the risk of eliciting allergic reactions. Our preliminary studies in a murine model of PN-induced anaphylaxis showed that the effect of subcutaneously (sc) administered engineered proteins was partially protective (Li et al, unpublished data). Other investigators demonstrated that bacterial adjuvants, such as heat-killed *Listeria monocytogenes* (HKLM), shifted antigen-specific T_H2 responses to T_H1-like responses and enhanced the response to allergen immunotherapy in a murine model of allergic asthma.[10] Recently, we demonstrated that the sc coadministration of HKLM and engineered PN proteins (HKLM-mAra h123) in PN-sensitized mice significantly reduced plasma histamine levels and anaphylactic symptom scores after intragastric (ig) PN challenge compared with sham-treated mice. IgE levels were significantly reduced, and IgG2a levels were significantly increased.[11] Because our engineered PN proteins are generated in *Escherichia coli,* which itself might serve as an adjuvant to promote a T_H1 response, we investigated the efficacy of a mixture of heat-killed *E coli* (HKE) producing mAra h1, 2, and 3 (HKE-MP123) and found that administering this mixture sc or rectally (pr), but not ig, suppressed PNA (Li et al, manuscript in preparation). The sc route of HKE-MP123 was abandoned, because it induced skin inflammation (Li et al, unpublished data) and was unlikely to be acceptable for human use. Because the rectal mucosa is heavily colonized with *E coli* and thousands of other organisms, it was believed that the pr administration of HKE-MP123 could provide an acceptable alternative. We recently compared the effects of pr administration of HKE-MP123 with a mixture of the 3 purified modified PN allergenic proteins (MP-123) and found that HKE-MP123, but not MP-123, suppressed PN-allergic reactions and did not induce local inflammatory reactions (Li et al, manuscript submitted for publication).

In this study, we examined the long-term protective effect of pr HKE-MP123 in the treatment of PN-allergic mice. PN-allergic C3H/HeJ mice received various doses of HKE-MP123 or vehicle alone (sham) and then were challenged on several occasions over a prolonged period. The medium and high doses of HKE-MP123 were found to provide the best protection. This novel immunotherapeutic approach seems to provide long-lasting protection and a broad immunoregulatory effect, which might benefit PN-allergic patients.

METHODS

Mice and reagents

Five-week-old female C3H/HeJ mice were purchased from the Jackson Laboratory (Bar Harbor, Me) and maintained on PN-free chow under specific pathogen-free conditions. Standard guidelines for the care and use of animals were followed.[12]

Freshly ground, whole-roasted PN and crude PN extract were prepared as previously described[13] and used as antigens. Cholera toxin (CT) was purchased from List Biological Laboratories, Inc (Campbell, Calif). Modified recombinant Ara h1, 2, and 3 were prepared as previously reported.[14] Concanavalin A and albumin-dinitrophenol (DNP-albumin) were purchased from Sigma (St Louis, Mo). Antibodies for ELISAs were purchased from the Binding Site Inc (San Diego, Calif) (sheep anti-mouse IgE, and biotinylated donkey anti-sheep IgG). Anti-DNP IgE and IgG2a were purchased from Accurate Scientific Inc (Westbury, NY).

Preparation of HKE-MP123

To prepare HKE-MP123, *E coli* clones BL21 (DE3) expressing mAra h1, mAra h2, and mAra h3, or carrying the pET24 (a)+ vector alone, were generated individually as previously described.[15] One hundred milliliters of medium containing 30 μg/mL of kanamycin was inoculated with a volume of the overnight bacterial culture containing approximately 2 optical units at 600 nm. The bacterial culture was grown at 37°C, ~240 rpm, for ≥ 2.5 hours until the optical density reached 0.6 optical units at 600 nm. Protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside at 1 mmol/L, and the bacteria were incubated for an additional 14 to 16 hours. The bacteria were inactivated by incubation in a water bath at 65°C for 30 minutes in aliquots of 50 mL or less, cooled on ice for ~20 minutes, and then collected by centrifugation. The bacteria were washed and resuspended in an appropriate volume of ice-cold PBS. The final HKE mixture containing approximately equal amounts of the 3 modified PN proteins was prepared. An equal amount of mAra-containing bacteria was used to produce HKE-MP123 (eg, high dose – 30 μg each of mAra h1, 2, and 3 = MP-123, total 90 μg protein), as measured by optical units at 600 nm and was used to prepare the modified PN protein mixtures and the HKE-carrying vector alone. The specific protein expression level was determined by comparing immunoblot analyses of the HKE-MP123 with serial dilutions of known quantities of bacterial cell lysates. The effectiveness of the heat-inactivation procedure was determined by plating bacterial aliquots taken from the induced culture and the final HKE suspensions on an LB-agar plate. Relative to the original induced bacterial cultures, less than 0.02% viable cells were detected in the final heat-treated bacterial suspensions. The integrity of the heat-treated bacteria was verified by analyzing the specific protein content in both the bacterial cell pellet and in the culture supernatant obtained by centrifugation (4000*g*). An immunoblot of the aliquot separated on an SDS polyacrylamide gel was prepared and was probed with either human polyclonal serum IgG antibodies (for modified Ara h1 and

Ara h3), or with an anti-modified Ara h2 mouse polyclonal IgG antibody. More than 99% of the specific recombinant protein was shown to be present in the bacterial cell pellet fraction.

Ig antigen sensitization, challenge, and HKE-mAra h1 to h3 treatment

Mice were sensitized with PN and CT as previously described.[11] As depicted in Fig 1, treatment began at week 10 after the initial PN sensitization. Six groups (n = 12/group) were involved: Group 1 (sham) received the methylcellulose vehicle; group 2 received HKE-MP123, 0.9 μg (low dose); group 3 received HKE-MP123, 9 μg (medium dose); and group 4 received HKE-MP123, 90 μg (high dose); group 5 received HKE-V (vector alone); and group 6 was neither sensitized nor treated (naive). Treatments with HKE were administered in 90 μL of methylcellulose as vehicle pr 3 times at weekly intervals. Mice were challenged ig 2, 6, and 10 weeks after therapy (weeks 14, 18, and 22 after initial sensitization). After each challenge, 4 mice were killed to collect samples for immunologic studies.

Assessment of hypersensitivity reactions

As shown in Fig 2, PN-sensitized mice given ig whole PN experienced anaphylactic reactions. Anaphylactic symptoms were evaluated 30 to 40 minutes after the second challenge dose using a scoring system as previously described[13]: 0 = no symptoms; 1 = scratching and rubbing around the snout and head; 2 = puffiness around the eyes and snout, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3 = wheezing, labored respiration, cyanosis around the mouth and the tail; 4 = no activity after prodding, or tremor and convulsion; 5 = death. Scoring of symptoms was performed in a blinded manner.

Measurement of plasma histamine levels

Plasma histamine levels were obtained 30 minutes after the second ig challenge dose and analyzed using an enzyme immunoassay kit (ImmunoTECH Inc., Marseille, France) as described by the manufacturer.[16]

Measurement of serum PN-specific IgE and IgG2a levels

Venous blood samples were obtained from the tail vein throughout sensitization/boosting, 1 day before treatment, during treatments, and 1 day before challenge. Sera were collected and stored at −80°C until analyzed. Levels of PN-specific IgE and IgG2a levels were determined as previously described.[13,17] Plates were coated with crude PN extract, incubated overnight at 47°C, and then blocked and washed. Serum samples (1:10 dilutions) were added to the plates, incubated overnight at 4°C, and then the plates were washed thoroughly. To determine IgE levels, sheep anti-mouse IgE (0.5 μg/mL) was added to the wells, incubated for 1 hour, and the plates were again washed thoroughly. Finally, biotinylated donkey anti-sheep IgG (0.5 μg/mL) was added to the wells and incubated at room temperature for 1 hour. After thorough washing, avidin-peroxidase was added for an additional 15 minutes at room temperature. For IgG2a measurements, biotinylated rat anti-mouse IgG2a monoclonal antibodies (0.25 μg/mL) were used as the detection antibodies. Subsequent steps were the same as those in IgE measurement. Equivalent concentrations of PN-specific IgE and IgG2a were calculated by comparison with a reference curve generated with anti-DNP IgE and IgG2a mouse monoclonal antibodies, as described previously.[13,17] Serum PN-specific IgE values were calculated by comparison with a reference curve generated with anti-DNP IgE mouse monoclonal antibodies, as described previously.[13,17]

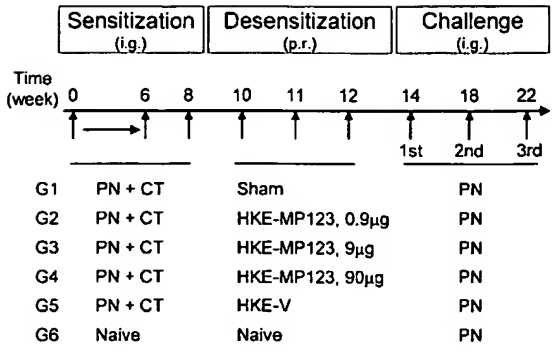

FIG 1. Experimental protocol. C3H/HeJ mice were sensitized intragastrically with freshly ground, whole roasted peanut together with cholera toxin over an 8-week period. Treatment was initiated at week 10. Six groups (n = 12/group) were involved: Group 1 (sham) received methylcellulose vehicle only; group 2 received HKE-MP123 0.9 μg (low dose); group 3 received HKE-MP123, 9 μg (medium dose); group 4 received HKE-MP123, 90 μg (high dose); group 5 received HKE-vector; and group 6 was naive. The treatments were administered rectally in methylcellulose as vehicle 3 times at weekly intervals. Mice were challenged 2, 6, and 10 weeks after the termination of therapy (week 14, 18, and 22, respectively, after desensitization). After each challenge, 4 mice were killed for collection of blood and tissue samples.

Cell culture and cytokine measurements

Immediately after evaluating anaphylactic reactions, 4 mice from each group were killed and splenocytes (SPCs) were isolated and cultured in RPMI 1640 containing 10% FBS, 1% penicillin/streptomycin, and 1% glutamine. SPCs were cultured in 24 well plates (4×10^6/well/mL) in the presence or absence of crude PN extract (50 μg/mL) or concanavalin A (2 μg/mL). Supernatants were collected after 72 hours of culture, and aliquots were stored at −80°C until analyzed. IFN-γ, IL-4, IL-5, IL-13, IL-10, and TGF-β levels were determined by ELISA according to the manufacturer's instructions (R&D systems, Minneapolis, Minn, for IL-13, PharMingen, San Diego, Calif, for all others).

Statistical analysis

Data were analyzed using SigmaStat statistical software package (SPSS Inc, Chicago, Ill). For histamine, IgE, and cytokine levels, the differences between the groups were analyzed by 1-way ANOVA followed by Bonferroni's t test for all pairwise comparisons, because the data passed the normality test. For symptom scores, the differences between the groups were analyzed by Kruskal-Wallis 1-way ANOVA on ranks followed by all pairwise comparison procedure (Dunn's), because the data failed to pass the normality test. P values < .05 were considered significant.

RESULTS

HKE-MP123 confers long-lasting protection against PN-induced anaphylaxis after oral PN rechallenge

To determine whether HKE-MP123 can provide a long-lasting effect on PNA, PN-sensitized mice were treated with 3 different weekly doses of pr administered HKE-MP123 in a methylcellulose carrier. Mice were then challenged ig with PN 2 weeks later (week 14 after

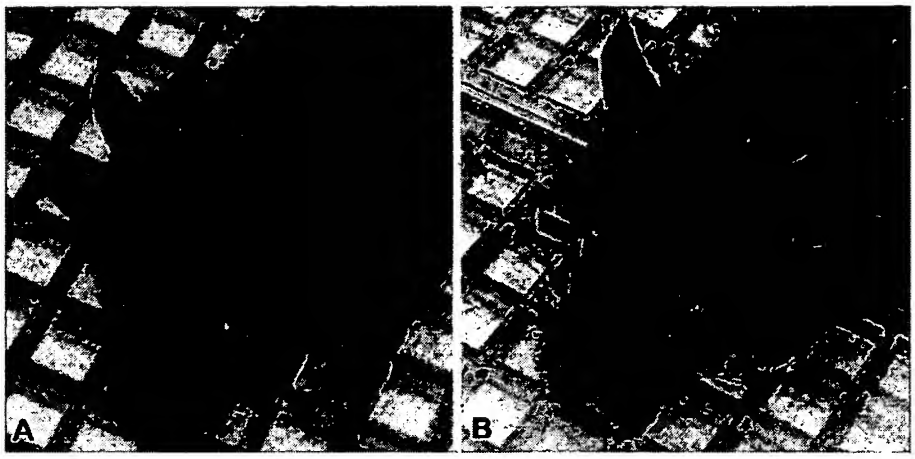

FIG 2. Mouse experiencing anaphylaxis. A, Normal C3H/HeJ mouse. B, Peanut-sensitized C3H/HeJ mouse experiencing an anaphylactic reaction after intragastric feeding of peanut; note swelling about the eyes and snout, pilar erecti, and cyanosis of the ears and feet.

the initial sensitization) and again 4 and 8 weeks later (weeks 18 and 22, respectively, after the initial sensitization). Anaphylactic symptom scores were evaluated 30 minutes after challenge. After the first challenge, all 3 HKE-MP123–treated groups exhibited significantly lower anaphylactic symptom scores compared with the sham-treated group (low-, medium-, and high-dose HKE-MP123–treated groups versus sham: $P < .01$, .01, and .01, respectively, Fig 3, *A*). No dose-response difference was observed among the HKE-MP-123–treated groups at the time of the first challenge. Surprisingly, HKE-V also significantly reduced symptom scores compared with the sham-treated group ($P < .05$), although the symptom scores tended to be greater in this group compared with the HKE-MP–123 treated groups. After the second challenge (week 18), anaphylactic symptom scores were reduced significantly only in the medium-dose and high-dose HKE-MP-123–treated groups ($P < .05$ and $P < .01$, respectively, Fig 3, *B*). Similarly, at the third challenge (week 22), only mice receiving the medium and high doses of HKE-MP-123 were protected from anaphylactic reactions ($P < .01$, Fig 3, *C*). These results demonstrate that higher doses of HKE-MP123 in a methylcellulose carrier resulted in persistent protection lasting at least 10 weeks.

HKE-MP123 has a long-lasting inhibitory effect on PN-induced histamine release

Because histamine is associated with the anaphylactic reactions, we also measured plasma histamine levels 30 minutes after PN challenge. We found that after the first challenge at week 14, plasma histamine levels were significantly reduced in all 3 HKE-MP-123–treated groups compared with the sham-treated group ($P < .01$, Fig 4, *A*). Interestingly, reduction of plasma histamine levels in the HKE-V–treated group failed to reach statistical significance. After the second challenge, only the high-dose HKE-MP123–treated group had significantly lower plasma histamine compared with the sham-treated group ($P < .01$, Fig 4, *B*). After the third challenge, histamine levels were significantly lower in both the medium-dose and high-dose-HKE-MP123–treated groups compared with sham-treated group ($P < .01$, Fig 4, *C*). Mice treated with a low dose of HKE-MP-123 and HKE-V did not show a significant reduction in plasma histamine levels compared with the untreated group after the second and the third challenge (Fig 4). These results parallel the clinical findings in that HKE-MP123 (at medium and high doses) has a long-lasting suppressive effect on histamine release, which lasted for at least 10 weeks

HKE-MP123 has a long-lasting inhibitory effect on PN-specific IgE production

PN-specific IgE levels were monitored during sensitization/boosting, desensitization, and after treatment. IgE levels increased markedly over the 8-week sensitization/boosting in each group of mice after PN sensitization and were similar among the groups before treatment at week 10. After treatment, IgE levels were significantly reduced in all HKE-MP123–treated groups at the first, second, and the third challenge ($P < .001$, Fig 5, *A*), being lowest in the high-dose treatment group. IgE levels were also reduced in the HKE-V–treated group at the time of the third challenge ($P < .05$) but were significantly greater than in the high-dose HKE-MP123–treated group ($P < .05$). IgG2a levels were significantly increased in the HKE-MP123 medium and high dose–treated groups at the first ($P < .01$ and $P < .001$ reactively), the second ($P < .05$ and $P < .01$ receptively), and the third challenges ($P < .05$ and .001 respectively) compared with the sham-treated group (Fig 5, *B*). IgG2a levels in the low-dose HKE-MP123–treated group were also significantly greater than in the sham-treated group at the time of the first and second challenge, but not at the third challenge. IgG2a levels in the HKE-V–treated group were not significantly different than those of the

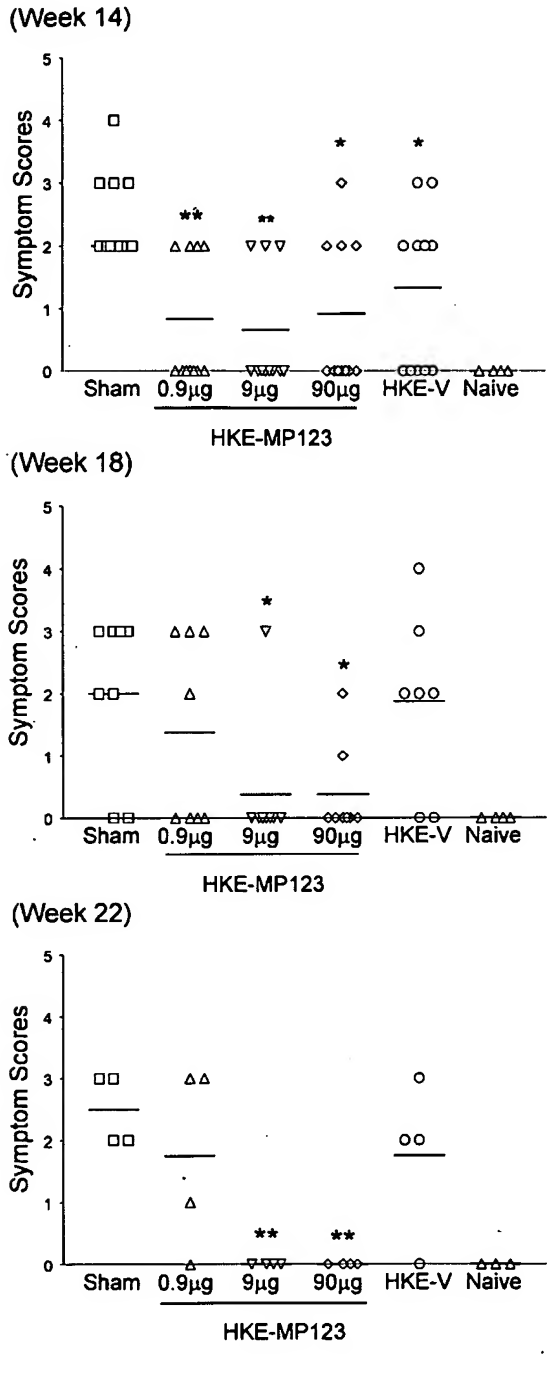

FIG 3. Persistent protection against peanut-induced anaphylactic reactions by HKE-MP123. Mice were challenged at weeks 2 (A), 6 (B), and 10 (C) after the last HKE-MP123 treatment. Anaphylactic symptom scores were determined 30 minutes after challenge. Each point indicates an individual mouse. *Bars* indicate the median of 12 mice (A), 8 mice (B), and 4 mice (C) in each group. $*P < .05$, and $**P < .01$ vs sham.

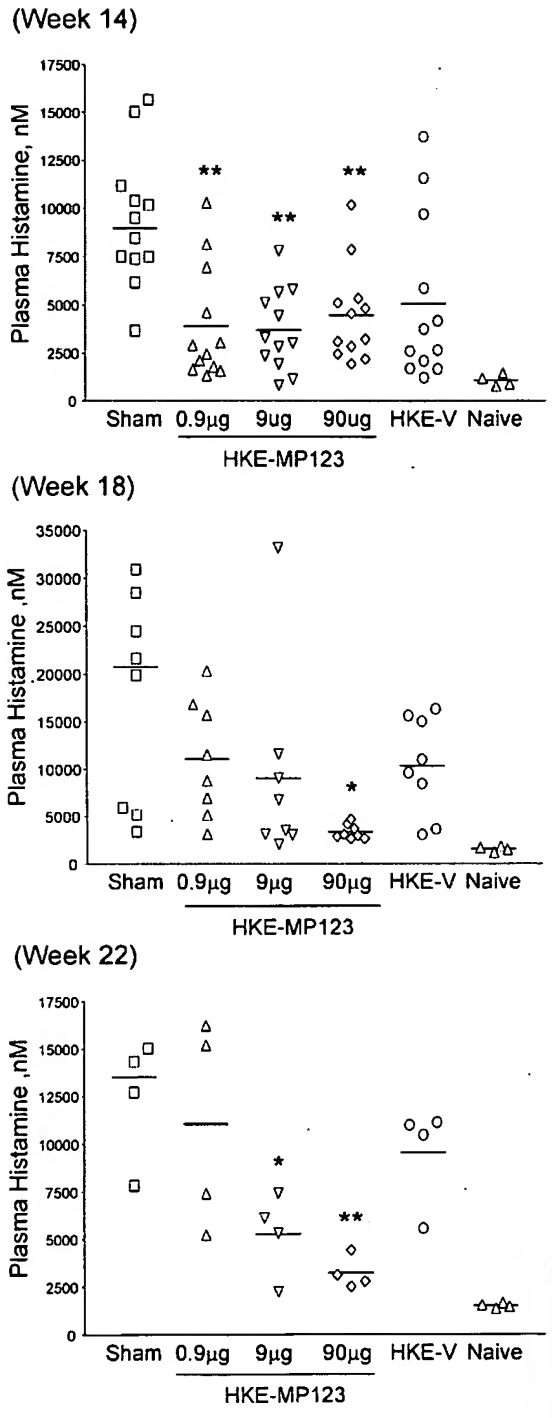

FIG 4. Effect of therapy on plasma histamine levels. Thirty to 40 minutes after challenge, blood was collected and plasma was obtained. Histamine levels were measured by use of an enzyme immunoassay kit. Data are means ± SEM for each group of 12 mice (A), 8 mice (B), and 4 mice (C) in each group. $*P < .05$, and $**P < .01$ vs sham.

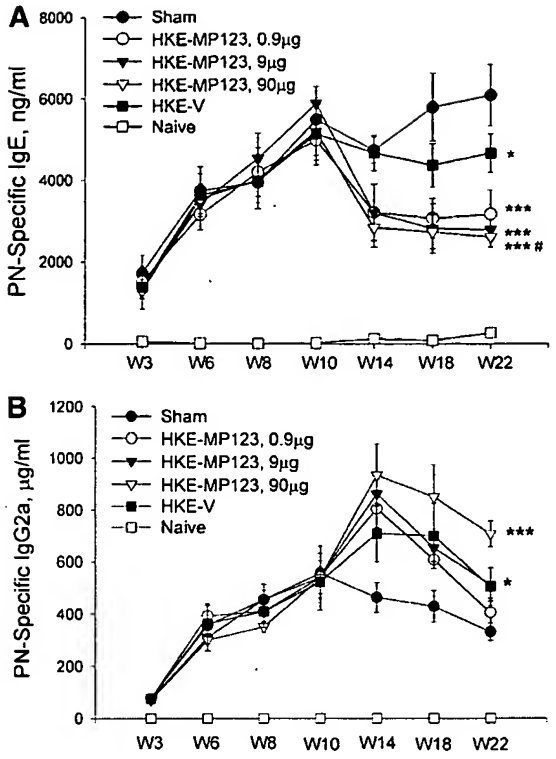

FIG 5. Effect of therapy on peanut-specific IgE levels. Sera from all groups of mice were obtained during sensitization (3, 6, and 8 weeks), before treatment, and 1 day before each challenge (weeks 14, 18, and 22). Peanut-specific IgE (**A**) and IgG2a (**B**) levels were determined by ELISA. Data are mean ± SEM for each group as in Fig 2. $*P < .05$ vs sham; $***P < .001$ vs sham.

sham-treated group at the time of the first and the second challenge. These results indicate that HKE-MP123 suppresses IgE and increases IgG2a production. This effect lasted at least 10 weeks after discontinuing therapy, and the high doses of HKE-MP123 seemed to be the most effective.

HKE-MP123 modulation of T_H1, T_H2, and T-regulatory cytokines

To determine whether the HKE-MP123–mediated long-lasting protection against PNA was associated with altered SPC cytokine profiles, we analyzed cytokine levels in SPC culture supernatants from each group of mice after the last challenge. IL-4 levels were significantly lower in the low-, medium-, and high-dose HKE-MP123–treated and HKE-V–treated groups compared with the sham-treated group ($P < .01$, .05, .01, and .05, respectively; Fig 6, *A*). IL-13 levels also were significantly decreased in the medium-dose and high-dose HKE-MP123 groups and the HKE-V group ($P < .01$, Fig 6, *B*). However, significant reduction of IL-5 levels was only seen in the medium and high HKE-MP123–treated groups ($P < .001$, Fig 6, *C*). IFN-γ levels were increased in all HKE-MP123–treated and HKE-V–treated groups ($P < .01$, .001, .001, and .001, respectively; Figs 5 and 6). IL-10 is a classic T_H2 cytokine believed to be involved in the induction of oral tolerance[18] and the downregulation of the allergic response.[19] TGF-β is also believed to be important in the development of oral tolerance to food allergens.[20] We found that IL-10 was reduced in all HKE-MP123–treated and HKE-V–treated groups compared with the sham-treated group, being lowest in the HKE-MP123 high-dose–treated group ($P < .01$, Fig 6, *E*). In contrast, TGF-β levels were significantly increased only in the HKE-MP123–treated groups in a dose-dependent manner ($P < .05$, $P < .01$, and $P < .01$; Fig 6, *F*). These results demonstrate that HKE-MP123 has a broad immunoregulatory effect on T_H1, T_H2, and T-regulatory cytokines, which might contribute to its beneficial effect on PNA.

DISCUSSION

PNA is a life-threatening food allergy for which there is no satisfactory treatment. Monthly injections of humanized recombinant anti-IgE antibodies, which reduces mast cell and basophil-bound IgE, seem to be effective in reducing allergic responses in PN-sensitive subjects, at least to small amounts of PN protein.[21] However, this treatment cannot "cure" food allergy, and continuous monthly injections are necessary to maintain protection. Consequently, additional therapeutic approaches for the treatment of food allergy are needed.

In the past several years, we have focused on developing novel immunotherapeutic approaches for PNA. In 1 approach, we have evaluated the efficacy of "engineered" recombinant protein-based immunotherapy in our murine model of PN-induced anaphylaxis.[11,22] The IgE-binding sites on 3 major PN allergens, Ara h1 to h3, were identified and altered by a single amino acid, MP-123. This change led to marked abrogation or elimination of IgE binding without significantly affecting the T-cell activating capacity of these "engineered" proteins. Although intranasal or sc administration of the engineered proteins partially desensitized PN-allergic mice, leading to a marked decrease in anaphylactic symptoms after PN challenge, the addition of bacterial products (eg, *L monocytogenes* or *E coli*) induced more substantial protection. Recently, we found that administering HKE expressed the engineered proteins, HKE-MP123 significantly reduced anaphylactic reactions, and pr administration seemed to be safe and highly efficacious in the murine model of PNA (Li et al manuscript, submitted for publication). In this study, we have demonstrated that 3 pr treatments with HKE-MP123 at medium (9 μg) or high doses (90 μg) provided PN-allergic mice with significant protection from anaphylaxis for at least 10 weeks after the discontinuation of therapy. Low-dose (0.9 μg) HKE-MP123 and HKE-V alone induced temporary protection (ie, protection against the first challenge) but not subsequent challenges. These results demonstrate that the pr administration of HKE-producing engineered PN proteins might be efficacious for treating PNA.

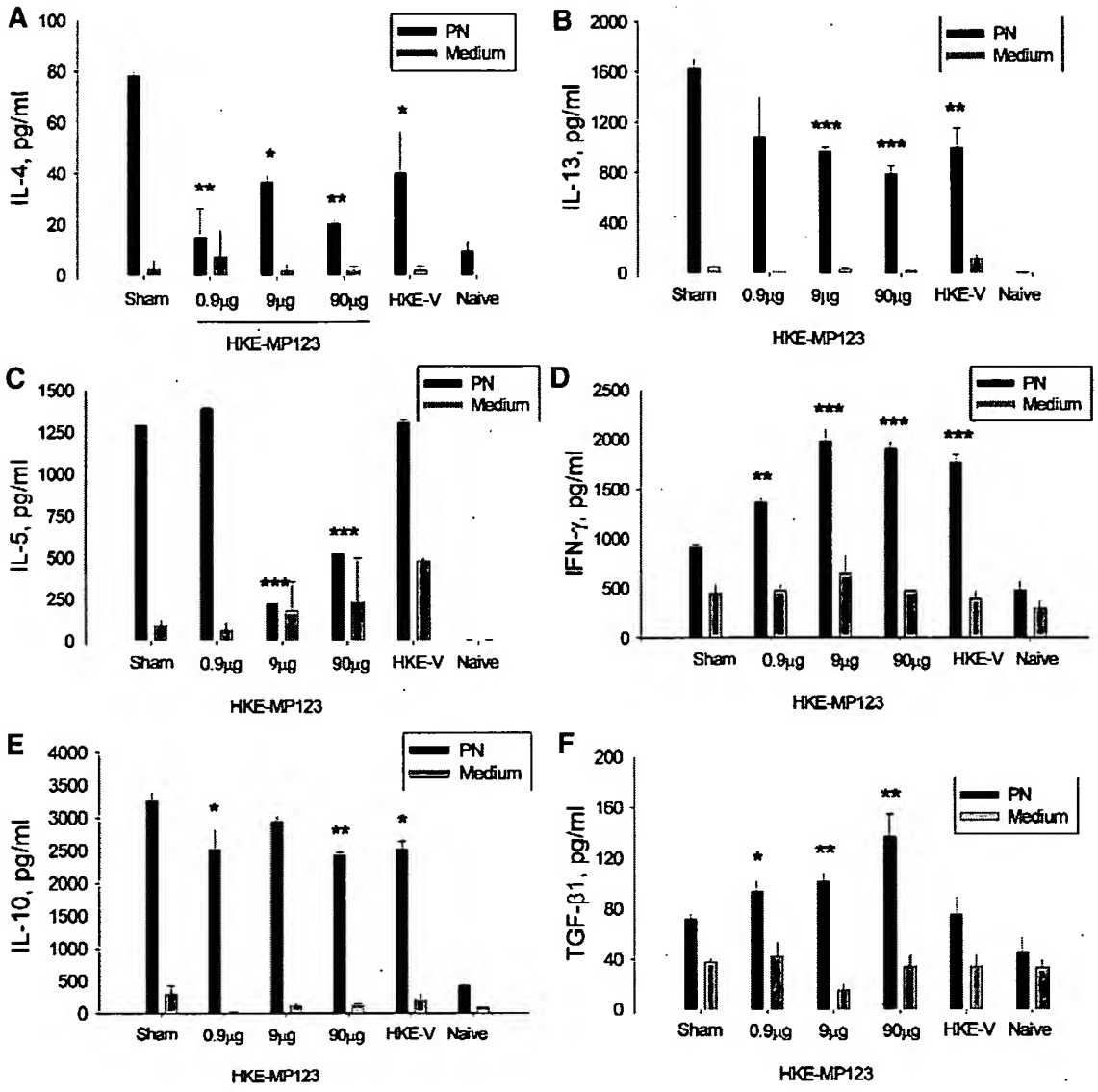

FIG 6. Effect of therapy on cytokine production. Cell suspensions were cultured in complete culture medium in the presence of crude peanut extract, medium alone, or concanavalin A (data not shown). Supernatants were collected 72 hours later, and cytokine levels were determined by ELISA. Results are expressed as mean ± SEM of 2 duplicate cultures of 4 mice. $*P < .05$; $** P < .01$,$***P < .001$ vs sham.

In addition to suppressing clinical symptoms, we found that HKE-MP123 produced the long-lasting suppression of histamine release after PN challenge and a decrease in PN-specific IgE levels. Interestingly, PN-specific IgE levels were also reduced in the HKE-V–treated group but were significantly greater than that seen in the high-dose HKE-MP123–treated group. This might have been due to the effect of CpG motifs in the plasmid vector. IgE levels were not significantly different in mice treated with the different doses of HKE-MP123, suggesting that the reduction in IgE is not solely responsible for the long-lasting protection mediated by HKE-MP123. IgG2a levels were significantly increased for at least 10 weeks in the medium-dose and high-dose HKE-MP123–treated groups and were associated with the long-lasting protection in these 2 groups. IgG2a, a T_H1-driven antibody[23-26] generally considered to be a "blocking antibody,"[27] was enhanced by HKE-MP123 treatment and might have been at least in part responsible for the long-lasting beneficial effect of immunotherapy in this model.

Numerous studies have demonstrated that T_H2 cytokines play a central role in the pathogenesis of allergic disorders, including food allergy. IL-4 and IL-13 promote B cell switching to IgE production and mast cell

activation, whereas IL-5 has been shown to have a potentially autocrine effect on mast cells, in addition to its recognized paracrine effects on eosinophils.[28,29] IFN-γ, on the other hand, inhibits T_H2 cell activation and mast cell/basophil mediator release on re-exposure to antigen.[30,31] Schade et al[32] recently demonstrated that T-cell clones generated from infants with cow milk allergy produced high levels of IL-4, IL-5, and IL-13 and low levels of IFN-γ, whereas T-cell clones produced from infants without cow milk allergy had high levels of IFN-γ and low levels of IL-4, IL-5, and IL-13. In addition, decreased IFN-γ was correlated with increased IgE levels in PN-allergic patients, and T_H2 clones have been generated from patients with PNA.[33,34] Allergen-based immunotherapies are believed to re-establish immunologic tolerance to allergen by redirecting T-cell immune responses from a T_H2-type to a T_H1-type response.[35] In this study, we found that 10 weeks after therapy, SPCs from mice treated with the higher doses of HKE-MP123 induced significant reductions of T_H2 cytokines and increases in IFN-γ, suggesting a shift from T_H2 responses to T_H1 responses. The low-dose HKE-MP123–treated group and the HKE-V–treated group both showed induction of IFN-γ and selective suppression of IL-4 and/or IL-13 but no effect on IL-5 production. These results suggest that higher doses of HKE-MP123 are more effective in regulating T_H1 and T_H2 responses, which might be associated with the long-lasting therapeutic effect on PNA.

Although the counterregulatory effect between T_H1 and T_H2 responses remains an important paradigm, an appreciation of the regulatory role of TGF-β, a T_H3 cytokine, and IL-10, defined as a T-suppressor cytokine, has developed for both T_H1-mediated autoimmune and T_H2-mediated allergic responses.[18,19,36-38] Colostrum TGF-β concentrations were found to be lower in samples from mothers of infants with IgE-mediated cow milk allergy than in samples from mothers of infants with non-IgE mediated cow milk allergy.[39] A recent study found that IL-10 was essential in parasite infection–mediated protection against PNA in a murine model.[40] However, any relationship between TGF-β, IL-10, and allergen immuno-therapy–mediated regeneration of oral tolerance to food antigen has not been demonstrated. In this study, we found that TGF-β levels were significantly increased in all HKE-MP123–treated groups but not the HKE-V–treated group and seemed to be dose dependent. These results suggest that the induction of TGF-β might also be important for the long-lasting therapeutic benefit of HKE-MP123 on PNA in this model. In addition, we found that IL-10 levels were reduced in all 3 HKE-MP123–treated and HKE-V–treated groups compared with the sham-treated group, being lowest in the high-dose HKE-MP123–treated group. These results suggest that IL-10 might play a less significant role in the HKE-MP123–mediated protective effect on PNA. We and others recently found that increased IL-10 production seemed to be associated with induction of PNA in this model.[40,41] In a study that used the coadministration of HKLM and ovalbumin in ovalbumin-sensitized mice, the suppression of IL-4 and increase in IFN-γ production was associated with a reduction of IL-10.[10] The key cytokine(s) and cellular mechanisms responsible for the long-lasting protection against PN anaphylaxis induced by HKE-MP123 in this study are unknown. Further work is needed to determine whether IFN-γ and/or TGF-β are the key cytokines in re-establishing the long-lasting oral tolerance meditated by HKE-MP123, to define the effector T cells, and to determine which antigen presenting cells are involved in initiating the immunoregulatory responses.

Of interest, the HKE-V treatment also induced statistically significant protection (although less than HKE-MP123) at the first challenge, which might be due to vector CpGs within the *E coli*, resulting in switching the T_H2 response to a T_H1 response. However, the HKE-V effect on PN allergy is unlikely attributable to the vector alone, because mock DNA (plasmid DNA alone) had essentially no effect on allergy in our previous study.[42]

In conclusion, we have demonstrated that the pr administration of high-dose HKE-MP123 has a potent and persistent therapeutic effect on PNA in this model of PN hypersensitivity. Protection lasted for at least 10 weeks and was accompanied by persistent reduction of PN-specific IgE and plasma histamine levels after challenges. The precise mechanisms associated with this long-lasting protection are not fully understood, but our study suggests that the protective effect is likely related to the downregulation of T_H2 cytokines, perhaps resulting from upregulation of IFN-γ and TGF-β. These studies suggest that in the future, pateints who are allergic to PN might be successfully "desensitized" by use of a suppository-like form of HKE-MP123.

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